Journal of the Egyptian Society of Haematology & Research, Vol. 5, No. 2, September: 41-47, 2009

Serum Soluble TNF-Related Apoptosis-Inducing Ligand (sTRAIL) Levels in Chronic Myeloid Leukemia Patients Receiving Imatinib Mesylate; Clinical importance

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Abstract

Background: Imatinib mesylate is now the first choice treatment for all newly diagnosed chronic myeloid leukemia (CML) patients. Despite the impressive percentage of responding patients, some CML cases show primary resistance or relapse after an initial response. Resistance to imatinib is commonly associated with reactivation of BCR-ABL signaling. Recently evidence has accumulated of the high induction of TRAIL expression in neutrophils when cells are stimulated with IFNs.

Objectives: The aim of the present work was to study the role of TRAIL in overcoming imatinib resistance in CML patients using combined therapy of imatinib mesylate and interferonα.

Material and Methods: In this study, we estimated the level of serum sTRAIL in fifty Ph+ve CML patients treated with the tyrosine kinase inhibitor; imatinib mesylate. They were divided into two groups according to the response to therapy after 6 months of follow up by RQ - PCR. Resistant cases were subjected to a combination therapy of imatinib+INFα for another 6 months and sTRAIL levels were re-evaluated.

Results: sTRAIL initial levels were 127.22±9.56pg/ml. After 6 months of imatinib therapy; its levels were 121.30± 7.84pg/ml and 125.44±6.77 pg/ml in the responder group (41 patients) and in the imatinib resistant group (9 patients) respectively. These results were slightly lower than those in normal controls (157±16.31pg/ml). But sTRAIL levels increased in the resistant group after the combination therapy of imatinib+INFα to 220.44±32.61pg/ml which showed a statistical significance.

Conclusion: TRAIL could induce cell death in Ph+ve cell lines that were refractory to imatinib suggesting that combination therapy of imatinib+INFα could be an effective strategy for the treatment of imatinib resistant CML cases.

Key Words: *Chronic myeloid leukemia – Imatinib mesylate – TRAIL – INFα.*

Introduction

Chronic myelogenous leukemia (CML) is a clonal myelopro-liferative expansion of transformed hematopoietic progenitor cells which is characterized by the Philadelphia chromosome (Ph1) that is created from the t (9;22) (q34;q11) **[1,2]**. The Ph1 results in the juxtaposition of BCR and ABL genes thus generating a 210 KDa chimeric protein termed BCR-ABL with marked tyrosine kinase activity **[1]**. Imatinib mesylate has been developed for the targeted inhibition of the Abelson kinase (c-ABL) **[3]** which represents the causative pathogenetic event for CML. Although imatinib mesylate has revolutionized the treatment of CML, **[4]** it does not represent curative eradication of the malignant clone in all cases **[5,6]**. Hence, the need to find potentially curative therapies remains. One possible strategy may be immunotherapy.

Tumor necrosis factor (TNF) related apoptosis-inducing ligand (TRAIL) is a pro-apoptotic member of the TNF super-family, involved in cell homeostasis and immune tumor surveillance **[7]**. Although it is primarily expressed as a type II trans-membrane protein, it may also exist as a biologically active soluble form (sTRAIL) that is generated through enzymatic shedding or released in association with microvesicles **[8]**. A number of studies have shown that both the membrane bound and the soluble form of TRAIL can induce apoptosis in many tumor cell lines **[9,10]**. TRAIL exerts its activity by interacting with a complex system of cell-surface receptors; 2 death receptors (DR4/TRAIL-R1 and DR5/TRAIL-R2) and 3 decoy receptors (DCR1/TRAIL-R3. DCR2/TRAIL-R4 and os-

teoprotegerin) **[9]**. Only DR4 and DR5 contain a functionally active cytoplasmic death domain that allows an apoptotic response upon TRAIL stimulation **[11]**. On the other hand, the 3 decoy receptors lack a functional death domain so they cannot transduce a pro-apoptotic signal; instead they compete with DR4, DR5 for TRAIL binding **[12]**. Thus the selective expression of these receptors on normal cells is regarded as one of the mechanisms for the preferential pro-apoptotic action of TRAIL against tumor cells but not normal cells **[12]**.

Interferon α (IFNα) has been used for more than 20 years **[13]** but it was not positioned as a curative therapy for CML; its role in the context of immune therapy has not been elucidated. The mechanisms of the anti-tumor action of IFNα in CML still remain vaguely understood. Evidence has accumulated of the high induction of TRAIL expression in neutrophils when cells are stimulated with IFNs **[14,15]**. IFNα enhances TRAIL expression on anti-CD3-stimulated human peripheral blood T cells. This is involved in their cytotoxic activities against renal cell carcinoma **[16]**. There are few reports that TRAIL selectively induced apoptosis in multiple myeloma cells **[17]** and some myeloid leukemia cells **[18]**, yet its role in anti-tumor immunity against hematological malignancies and the role of IFNα as an immunotherapeutic agent for CML remains to be elucidated. In the present work, we studied the role of sTRAIL in overcoming imatinib resistance in CML patients using combined therapy of imatinib mesylate and interferonα.

Material and Methods

Patients:

Fifty patients with CML presenting to the Medical Research Institute, and Alexandria Health Insurance Hospital were enrolled in the study during the period from January 2006 to December 2008. They were 22 males and 28 females, their ages ranged from 31 to 68 with a mean of 48.32±9.53 and a median of 47 years. All of them had Ph-positive CML. Ten healthy, age and sex matched, individuals were included as control. The study was conducted in accordance to the guidelines of the 1975 Declaration of Helsinki **[19]**. Each patient signed an informed consent forum prior to inclusion in the study. This study was also approved by the local ethical committee.

Exclusion criteria included patients below 18 years of age, prior treatments for CML (only hydroxyurea was allowed), Philadelphia chromosome negative patients and those with complex karyotypes. Cardiac patients, those with serum creatinine more than 2mg/dl and those with severe impairment of liver functions were also excluded.

Methods:

All patients were clinically examined and were subjected to complete blood picture **[20]**, bone marrow examination **[21]** and leucocyte alkaline phosphatase scoring **[22]**. Then, they were stratified according to Sokal scoring system **[23]** which adopts the following prognostic parameters: Older age, enlarged spleen, high platelet count and high peripheral blast percentage.

- Sokal score 1 = score <0.8.
- Sokal score 2 = score 0.8-1.2.
- Sokal score 3 = score >1.2.

An online calculation for Sokal staging is available at: http://www.nrhg.ncl.ac.UK/cgi-bin/cml/Sokal.pl.

BCR-ABL fusion gene detection was done by Real-time quantitative Polymerase Chain Reaction (RQ-PCR) **[24]**.

Serum soluble TRAIL was estimated by ELISA:

Concentrations of sTRAIL, in the sera of patients and controls were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (TRAIL/Apo2L ELISA KIT, Diaclone Research, Besancon, France), according to the manufacturer's instructions. All samples were tested in duplicate.

Study design:

- Serum sTRAIL estimation on initial diagnosis upon enrollment in the study.
- Imatinib mesylate therapy at a dose of 400-mg/day orally for 6 months.
- Assessment of hematological remission at 3 months & 6 months after initiation of imatinib therapy.
- Assessment of cytogenetic remission after 6 months from initiating imatinib therapy.
- Serum sTRAIL estimation after 6 months of imatinib therapy.
- Starting IFNα therapy at a dose of 3-5 million IU 3-5/w along with imatinib mesylate for

patients who were resistant to imatinib alone after 6 months.

- Serum sTRAIL re-estimation for the last group after 6 months of initiation of the combined therapy with IFNα and imatinib.

A complete hematologic remission is defined as the achievement of normal WBC and platelet counts and normal differential count as well as disappearance of all symptoms and signs of CML. A partial hematologic response is defined as a decrease in the WBC count to less than 50% of the pretreatment level, or the normalization of the WBC count accompanied by persistent splenomegaly or immature cells in the peripheral blood. A complete cytogenetic response is defined as the absence of Ph-positive metaphases in marrow cells, and partial cytogenetic response as 1% to 34% Ph-positive metaphases. Major cytogenetic remission combines the percentages of complete and partial response. A reduction of BCR-ABL mRNA by 3-log or more, compared with a standardized baseline, has been designated a major molecular response (MMR), whereas consistent lack of detection of BCR-ABL mRNA is referred to as a complete molecular response (CMR) **[25]**.

Statistical analysis of data was done using SPSS version 10.0 Student's *t*-test was used for statistical comparisons of means. Pearson's correlation coefficient was calculated to evaluate the correlation between sTRAIL values of each sample and hematologic parameters. *p*-value below 0.05 was considered significant.

Results

The peak incidence in CML patients included in the study was the fifth decade. The main presenting symptom was fatigue in 35 patients (70%) and the most frequent sign was splenomegaly in all patients (100%), the splenic size was measured in centimeters by ultrasonography.

Table (1) shows the main hematologic data of CML patients included in the study.

Serum sTRAIL:

The initial serum sTRAIL in newly diagnosed CML patients was 127.22±9.56pg/ml; these levels were slightly lower than those in normal controls (157±16.31pg/ml). Serum sTRAIL estimation after 6 months of imatinib therapy showed rather low levels than the initial samples; (121.30±7.84pg/ml) in the responder group and (125.44±6.77pg/ml) in the resistant group but the difference was not statistically significant. On the other hand, during IFNα + imatinib therapy, sTRAIL levels were significantly elevated (220.44±32.61pg/ml). The difference was statistically significant as compared with initial sTRAIL estimation and sTRAIL after imatinib therapy alone ($p<0.001$), ($p=<0.001$) respectively (Table 2).

Table (1): CML patients' characteristics at presentation (No.=50).

Parameter	Value
Age: years	48.32±9.53 (31-68)*
Sex: male/female: No. (%)	22/28 (44/56)
Hemoglobin level: g/dl	9.94±1.68 (6.8-12.8)*
Total leucocytic count: $x10^9/L$	113.30±72.48 (31-440)*
Platelet count: $x10^9/L$	316.32±163.05 (63-870)*
Peripheral blood blasts: %	2.44±2.10 (0-8)*
Basophils: %	4.10±2.05 (1-10)*
Eosinophils: %	1.50±1.11 (0-4)*
Bone marrow blasts: %	3.14±1.81 (0-8)*
Bone marrow basophils: %	4.56±1.96 (2-12)*
Sokal score: No. (%)	
Stage I	9 (18%)
Stage II	30 (60%)
Stage III	11 (22%)

* Mean ± SD (range)

Table (2): Serum sTRAIL levels in CML patients in relation to time of estimation and response to therapy as compared to control.

Group	Serum sTRAIL (pg/ml)	
	Mean±SD	Range
Control	157.00±16.31	130.00-175.00
TRAIL before treatment	127.22±9.56	105.00-140.00
TRAIL after imatinib therapy		
• Responders	121.30±7.84	106.00-135.00
• Resistant group	125.44±6.77	118.00-139.00
TRAIL after IFNα+ imatinib therapy	220.44±32.61	180.00-275.00
t1	3.34	
t2	8.35*	
t3	8.57*	
p	<0.001*	

t1 : Comparison between initial serum sTRAIL & serum sTRAIL after imatinib alone

t2 : Comparison between serum sTRAIL after imatinib & serum sTRAIL after imatinib+IFNα

t3 : Comparison between initial serum sTRAIL & serum sTRAIL after imatinib+IFNα

In addition, there was no statistically significant correlation between serum sTRAIL estimations and age, Hb concentration, platelet count, TLC, PB or BM blast %. Also serum sTRAIL did not correlate with Sokal stages of the patients included in this study (Fig. 1).

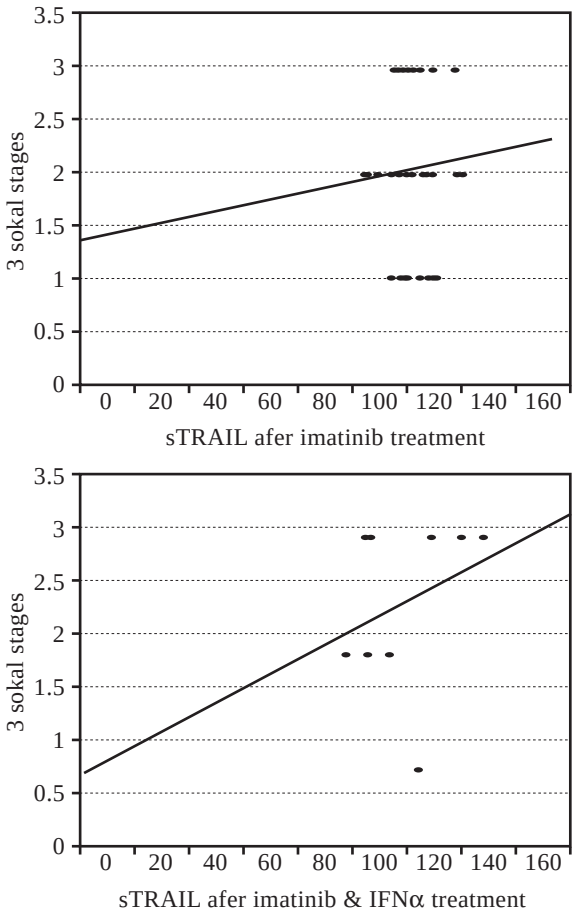

Fig. (1): Correlation between serum sTRAIL and the 3 Sokal stages, during imatinib therapy and during IFNα + imatinib.

Discussion

Evidence has accumulated of the high induction of TRAIL expression in neutrophils when cells are stimulated with IFNα and IFNγ **[9,14,15,26]**. Liu et al. **[27]** have shown that neutrophils obtained from CML-chronic phase (CP) patients had markedly increased TRAIL mRNA expression upon in vitro IFNα stimulation.

In this context, Tecchio et al. **[9]** reported that in vitro IFNα- activated neutrophils and monocytes were able to release a biologically active soluble form of TRAIL. IFNα-induced TRAIL protein is retained in the cytoplasm of neutrophils mainly in secretory vesicles and light membrane fractions which are then mobilized rapidly onto the cell surface or secreted as sTRAIL upon stimulation by pro-inflammatory mediators **[15]**.

In this study, we showed that serum sTRAIL levels were significantly elevated upon combined administration of IFNα and imatinib mesylate as compared to its levels at presentation or with imatinib mono-therapy in CML-CP patients. To the best of our knowledge, there are very few reports demonstrating that sTRAIL levels increase during IFNα administration in patients with neoplasia **[17,18]**. In this regard, it should be noted that this work demonstrated that the sTRAIL levels detectable in the newly diagnosed CML-CP patients were slightly lower than those in normal controls. This is in agreement with previous reports **[27,28]** showing that TRAIL mRNA expression was readily detectable but at a relatively low level in unstimulated CML neutrophils and in resting neutrophils in normal healthy subjects.

It should also be noted that sTRAIL estimation was done after 6 months of the combined administration of IFNα and imatinib denoting that the elevation of sTRAIL was not a transient phenomenon at the initiation of IFNα therapy but was maintained during the therapy. The present work also showed that sTRAIL levels during imatinib monotherapy were rather low as compared with the initial samples from newly diagnosed CML-CP patients but the difference did not reach statistical difference. This could indicate that the elevation of serum sTRAIL in our study is exclusively related to IFNα therapy and is not related to the clinical phase of CML or to the lowered burden of the CML clone. Furthermore, imatinib alone may not be participating in the TRAIL-mediated cell killing in these CML patients. This was the rationale for the use of IFNα in combination with imatinib in CML patients in this study.

It is noteworthy that, even the neoplastic neutrophils, such as those of CML which are transformed by the BCR- ABL fusion gene, retain the ability to produce cytokines as sTRAIL in the same manner as normal neutrophils **[28]**. Tecchio et al. **[9]** have shown that CML neutrophils and mononuclear cells, upon incubation with therapeutic doses of IFNα, release sTRAIL into the extracellular environ-

ment as efficiently as normal leucocytes. In contrast sFasL was not expressed or released by normal or CML neutrophils. Those latter authors could not obtain sera of CML patients subjected to IFNα therapy due to the current use of imatinib mesylate as the treatment of choice in CML. But in the current study, serum sTRAIL levels were reassessed in patients receiving imatinib+IFNα on the basis of the emergence of cases of imatinib resistance and the fact that the new tyrosine kinase inhibitors are still investigational which made the combined use of imatinib and IFNα feasible.

According to Tecchio et al. **[9]** and Plasilova et al. **[18]**, the leucocyte-derived sTRAIL would preferentially induce apoptosis of the CD34 Ph+ leukemic blasts rather than of leukemic neutrophils themselves. This is further supported by the fact that $CD34^+$ cells isolated from CML patients but not from healthy subjects express TRAIL-R1 and TRAIL-R2 death receptor **[29]**, which are absent on normal $CD34^+$ cells. Thus the soluble form of TRAIL released by IFNα-activated leucocytes can signal via both receptors and produce its pro-apoptotic effects. Another mechanism was proposed trying to explain how IFNα can suppress the leukemic clone in CML. It was suggested that, in Ph^+ leukemias, the immunomodulatory activity of IFNα might be mediated by the membrane-bound TRAIL expressed on activated T lymphocytes **[12]**. However, Tecchio et al. **[9]** have concluded that IFNα exerts its immunomodulatory activities through promoting the release of soluble TRAIL rather than by inducing the expression of the corresponding membrane-bound form.

It should be noted that, preliminary experiments revealed that concentrations of imatinib up to 500 nM do not interfere with the ability of IFNα to trigger the release of sTRAIL by either normal or CML leucocytes (C.T., M.A.C., unpublished data, January 2004) **[9]**. Furthermore, Uno et al. **[12]** have demonstrated that imatinib mesylate efficiently repressed most of the TRAIL-resistant cell lines, while TRAIL repressed most of the imatinib mesylate-resistant cell lines which suggests a potential clinical utility of TRAIL for patients with imatinib mesylate resistant CML. The correlation of this suggestion and the clinical response to IFNα and imatinib mesylate was the basis for this study.

In this context, Uno et al. **[12]** have demonstrated that all TRAIL-sensitive cell lines expressed the death-inducing receptor, DR4 and/or DR5 on their surface and none of the decoy receptors DCR1 and DCR2 whereas the TRAIL-resistant cell lines expressed neither DR4 nor DR5. In addition, regulators of the death signaling pathway such as caspase-8, FADD and FLIP are not critically involved in the determination of TRAIL sensitivity. It has been demonstrated that the binding of TRAIL to DR4 and DR5 as well as DCR2 induced the NF-kB activation and that the increased NF-kB activity protects against pro-apoptotic stimuli **[29]**. The pretreatment with NF-kB inhibitors enhanced sensitivity to TRAIL in TRAIL sensitive-cell lines, suggesting that the DR4/DR5-mediated or BCR-ABL mediated NF-kB activation plays a substantial role in protecting Ph-l positive leukemic cells from TRAIL-induced cell death. However, NF/kB inhibitors could not overcome TRAIL resistance in TRAIL-resistant cell lines not expressing DR4 or DR5 **[29]**.

Moreover, chemotherapeutic agents can increase the expression of those death receptors on tumor cells thus leading to enhanced killing on combining these agents with TRAIL as the extent of apoptosis by TRAIL is tightly regulated by the expression of these receptors and by downstream signaling **[30]**.

Ghaffari et al. **[31]** had shown that BCR-ABL suppression of TRAIL transcription is mediated through phosphorylation and inhibition of FOXO3 (FKHRL1); a transcription factor that functions down stream of BCR-ABL tyrosine kinase as a phosphorylated inactive form in CML. Imatinib mesylate induces cell cycle arrest and subsequent apoptosis via the conversion of FKHRL1 from the phosphorylated inactive form to the dephosphorylated active form in CML cell lines. Active FKHRL1 inhibits cell growth and cell cycle progression and subsequently induces apoptosis accompanied by up-regulation of TRAIL thus active FKHRL1 can overcome imatinib resistance in CML cells via TRAIL production. So, BCR-ABL suppression of TRAIL transcription may provide another mechanism for tumorigenicity in CML.

Moreover, BCR-ABL independent signaling pathways may contribute to imatinib resistance in some CML patients. Park et al. **[32]** demonstrated that the loss of BCR-ABL in imatinib

resistant cells led to the down regulation of c-FLIP and subsequent increase of TRAIL sensitivity suggesting that TRAIL could be an effective strategy for the treatment of this category of imatinib resistant patients as well.

In conclusion, our data indicates that IFNα can exert its immunomodulatory activities through promoting the release of sTRAIL and thus provides the rationale for the use of IFNα with imatinib mesylate for the treatment of Ph1-positive CML. Finally, in view of the lack of systemic toxic effects and synergistic activity of sTRAIL with other chemotherapeutic agents **[33]**, we recommend the use of recombinant TRAIL as an ideal substitute for IFNα in combinational regimens. Monoclonal antibodies against TRAIL death receptors in CML should be investigated as well.

References

1- Vardiman JW, Pierre R, Thiele J, et al. Chronic Myelogenous leukemia. In: Jaffe ES, Harris NL, Stein H, et al. Eds. World Health Organization Classification of tumors: pathology and genetics of tumors of hematopoietic and lymphoid tissues. Lyon, France: IARC Press. 2001, 20-26.

2- Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: The 2008 World Health Organization criteria and point-of-care diagnostic algorithms. Leukemia. 2008, 22: 14-22.

3- Deukwe BJ, Tamura S, Buchdunger E, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med. 1996, 2: 561-66.

4- Goldman JM. Chronic myeloid leukemia-still a few questions. Exp Hematol. 2004, 32: 2-10.

5- Bhatia R, Holtz M, Niu N, Gray R, et al. Persistence of malignant hematopoietic progenitors in chronic myelogenous leukemia patients in complete cytogenetic remission following imatinib mesylate treatment. Blood. 2003, 101: 4701-7.

6- Michor F, Hughes TP, Iwasa Y, Branford S, Shah NP, Sawyers CL, Nowak MA. Dynamics of chronic myeloid leukemia. Nature. 2005, 435: 1267-70.

7- Yagita H, Takeda K, Hayakawa Y, Smyth MJ, Okumura K. TRAIL and its receptors as targets for cancer therapy. Cancer Sci. 2004, 95: 777-83.

8- Monleon I, Martinez-Lorenzom J, Monteagudo L, et al. Differential secretion of Fas ligand or APO2 ligand/TNF-related apoptosis-inducing ligand-carrying microvesicles during activation-induced death of human T-cells. J Immunol. 2001, 167: 6736-44.

9- Tecchio C, Huber V, Scapini P, et al. IFNα-stimulated neurophils and monocytes release a soluble form of TNF-related apoptosis-inducing ligand (TRAIL/APO2 ligand) displaying apoptotic activity on leukemic cells. Blood. 2004, 103: 3837-44.

10- Kagawa S, He C, Gu J, Koch P, Rha SJ, Roth JA, Curley SA, Stephens LC, Fang B. Anti-tumor activity and by stander effects of the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) gene. Cancer Res. 2001, 61: 3330-8.

11- Wajant H, Pfizenmaier K, Scheurich P. TNF-related apoptosis inducing ligand (TRAIL) and its receptors in tumor surveillance and cancer therapy. Apoptosis. 2002, 7: 449-59.

12- Uno K, Inukai T, Kayagaki N, Goi K, et al. TNF-related apoptosis-inducing ligand (TRAIL) frequently induces apoptosis in Philadelphia chromosome-positive leukemia cells. Blood. 2003, 101: 3658-67.

13- Allan NC, Richards SM, Shepherd PC. UK Medical Research Council randomized, multi-centre trial of interferon-alpha n1 for chronic myeloid leukemia: Improved survival irrespective of cytogenetic response. The UK Medical Research Council's Working Parties for Therapeutic Trials in Adult Leukemia. Lancet. 1995, 345: 1392-97.

14- Kamohara H, Matsuyama W, Shimozato O, Abe K, et al. Regulatoin of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and TRAIL receptor expression in human neutrophils. Immunology. 2004, 111: 186-94.

15- Cassatella MA, Huber V, Calzetti F, Margotto D, Tamassia N, et al. Interferon-activated neutrophils store a TNF-related apoptosis-inducing ligand (TRAIL/Apo-2 ligand) intracellular pool that is readily mobilizable following exposure to pro-inflammatory mediators. J Leukoc Biol. 2006, 79: 123-32.

16- Kayagaki N, Yamaguchi N, Nakayama M, Eto H, et al. Type 1 interferons (IFNs) regulate tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression on human T cells: A novel mechanism for the anti-tumor effects of type 1 IFNs. J Exp Med. 1999, 189: 1454-60.

17- Chen Q, Gong B, Mahmoud-Ahmed AS, et al. APO2 L/TRAIL and Bcl-2- related proteins regulate type 1 interferon-induced apoptosis in multiple myeloma. Blood. 2001, 98: 2183-92.

18- Plasilova M, Zivny J, Jelinek J, et al. TRAIL (Apo2L) suppresses growth of primary human leukemia and myelodysplasia progenitors. Leukemia. 2002, 16: 67-73.

19- www.wma.net.

20- Howven B. The blood cell count: Rowan RM, Van Assen-defft OW, Perston EE (eds). Advanced laboratory methods in hematology. 1st ed., Great Britain. Library of Congress Cataloging. 2002, 2: 19-44.

21- Bates I. Bone Marrow Biopsy. In: Lewis Sm, Bain BJ and Bates I (Eds). Dacie and Lewis Practical Hematology, 10th ed. Great Britain. Library of Congress Cataloging. 2006, 6: 115-130.

22- Swirsky D, Bain BJ. Erythrocyte and leucocyte cytochemistry. In: Lewis SM, Bain BJ and Bates I (eds.). Dacie and Lewis Practical Hematology, 10th ed. Great Britain. Library of Congress Cataloging. 2006, 6: 115-130.

23- Sokal JE, Cox EB, Baccarani MB, et al. Prognostic discrimination in good risk chronic granulocytic leukemia. Blood. 1984, 63: 789-99.

24- Van Der Velden VH, Hochhaus A, Cazzaniga G, et al. Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: principles, approaches and laboratory aspects. Leukemia. 2003, 17: 1013-34.

25- Baccarani M, Saglio G, Goldman J. Evolving concepts in the management of chronic myeloid leukemia: Recommendations from an expert panel on behalf of the European Leukemia Net. Blood. 2006, 108 (6): 1809.

26- Koga Y, Matsuzaki A, Suminoe A, Hattori H, Hara T. Neutrophil derived TNF-related apoptosis-inducing ligand (TRAIL): a novel mechanism of anti-tumor effect by neutrophils. Cancer Res, 2004, 64: 1037-43.

27- Liu LG, Tanaka H, Ito K, Sultana TA, Kyo T, Kimura A. Absence of gene mutation in TRAIL receptor 1 (TRAIL-R1) and TRAIL receptor 2 (TRAIL-R2) in chronic myelogenous leukemia and myelodysplastic syndrome, and analysis of m-RNA expressions of TRAIL and TRAIL related genes in chronic myelogenous leukemia. Acta Haematol. 2005, 113: 113-23.

28- Tanaka H, Ito K, Kyo T, Kimura A. Treatment with IFN alpha in vivo up-regulates serum-soluble TNF-related apoptosis inducing ligand (s TRAIL) levels and TRAIL m RNA expressions in neutrophils in chronic myelogenous leukemia patients. Europ J Haematol. 2007, 78: 389-98.

29- Chaudhary PM, Eby M, Jasmine A, Bookwalter A, Murray J, Hood L. Death receptor 5, a new member of the TNFR family, and DR4 induce FADD-dependent apoptosis and activate the NF-kappaB pathway. Immunity. 1997, 7: 821-30.

30- Henson ES, Johnson JB, Gibson SB. The role of TRAIL death receptors in the treatment of hematological malignancies. Leuk Lymphoma. 2008, 49 (1): 27-35.

31- Ghaffari S, Jagani Z, Kitidis C, Lodish HF. Cytokines and BCR-ABL mediate suppression of TRAIL-induced apoptosis through inhibition of forkhead FOX3a transcription factor. Proc Natl Acad Sci U S A. 2003, 100 (11): 6523-28.

32- Park SJ, Kim MJ, Kim HB, Kang CD. Sensitization of imatinib- resistant CML cells to TRAIL- induced apoptosis is mediated through down- regulation of Bcr- Abl as well as c-FLIP. Biochem J. 2009, 420: 73-81.

33- Smyth MJ, Takeda K, Hayakawa Y. Nature's TRAIL- on a path to cancer immunotherapy. Immunity. 2003, 18: 1-6.