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TITLE: Contribution of Protein Tyrosine Phosphatases to the Ontogeny and Progression of Chronic Myeloid Leukemia

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<b>14. ABSTRACT</b> The JAK and STAT families of signal transduction molecules play critical role in the pathogenesis of chronic myeloid leukemias (CML). Inappropriate STAT1 and STAT5 activation have been observed in the Philadelphia chromosome-positive CML cell lines K562 and BV17, yet low levels of JAK1 tyrosine phosphorylation were observed suggesting that BCR/Abl directly tyrosine phosphorylates and activates STATs. The protein tyrosine phosphatases TC-PTP and PTP1B are negative regulators of JAK/STAT signaling molecules and it is possible that these two PTPs could impede the ability of CML cells to survive and proliferate in response to p210 BCR-Abl. We examined the role of TC-PTP and PTP1b in contributing to the CML phenotype and found that in some CML cell lines the levels of TC-PTP and PTP1b is increased suggesting that they may be potential caused of the reduced phosphorylation of the JAK kinases in CML.					
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## **General summary and context of funding.**

The grant award had for main task to demonstrate the potential role of PTPases in the development and potentially treatment of Chronic Myeloid Leukemias. It is important to note that although funds were approved at the U.S. Army Medical Research and Materiel Command in the spring 2004, because of various animal and biological ethic forms requirement, the funds were not available until spring 2005. This is in part the reason for our delay in moving forward with task three of our objectives and for submitting this report late. We apologize for the later.

## **Body of the report**

### **Introduction**

#### **Possible implications of TC-PTP and PTP1B in Chronic Myelogenous Leukemia**

Current treatments toward a cure for chronic leukemias using standard chemotherapeutic agents have reached their limits. Newer therapies that would target inappropriately activated cell-signaling pathways, which stimulate the uncontrolled growth and survival of cancer cells, are now being envisaged. CML is an excellent target for the development of such selective treatment as it is associated, in 90% of patients, with a genetic abnormality creating a fusion gene-product p210 BCR-Abl, also known as the Philadelphia chromosome (Tough, Court Brown et al. 1961; Caspersson, Gahrton et al. 1970; Shtivelman, Lifshitz et al. 1985). BCR-Abl has been shown to cause cellular transformation by activating signaling pathways normally triggered by the physiologic cytokines of hematopoietic cells (Steelman, Pohnert et al. 2004). The JAK and STAT families of signal transduction molecules are widely used by cytokine receptors to link receptor stimulation to gene transcription, leading to cellular growth, differentiation and proliferation. This pathway is crucial for normal hematopoietic development (Rane and Reddy 2002). Several groups have demonstrated the critical role of the JAK/STAT pathway in the pathogenesis of CML. Inappropriate STAT1 and STAT5 activation have been observed in the Philadelphia chromosome-positive CML cell lines K562 and BV173 (Carlesso, Frank et al. 1996; Ilaria and Van Etten 1996). However, only low levels of JAK1 tyrosine phosphorylation were observed, and JAK1 did not co-immunoprecipitate with BCR/Abl. These results suggest that BCR/Abl directly tyrosine phosphorylates and activates STATs (Ilaria and Van Etten 1996). Several PTPs play important roles in regulating hematopoiesis. In particular, TC-PTP and PTP1B are negative regulators of cytokine signaling, by dephosphorylating JAK/STAT signaling molecules activated upon cytokine receptor engagement (Haspel and Darnell 1999; McBride, McDonald et al. 2000; Myers, Andersen et al. 2001; Kaszubska, Falls et al. 2002; Simoncic, Lee-Loy et al. 2002; ten Hoeve, de Jesus Ibarra-Sanchez et al. 2002; Gu, Dube et al. 2003). Thus, it is possible that these two PTPs could impede the ability of CML cells to survive and proliferate in response to p210 BCR-Abl through their capacity to dephosphorylate constitutively activated proteins. TC-PTP and PTP1B have also been shown to modulate c-abl, its oncogene BCR-Abl, as well as signaling pathways that are influenced by these tyrosine kinases (LaMontagne, Flint et al. 1998 and Ibarra-Sanchez et al, in preparation; LaMontagne, Hannon et al. 1998).

### **Task 1: Development of the initial cell based models**

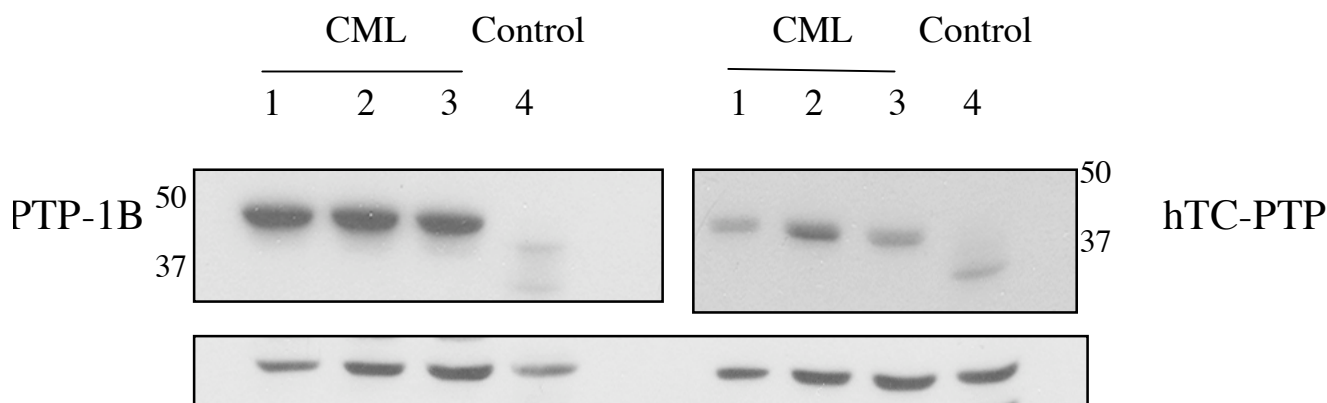
#### **Obtain and culture CML cell lines**

To evaluate the potential role of TC-PTP, PTP1B and PTP-PEST in the development of CML we have chosen to study the K562, MEG-01 and the KU812 human cell lines available as commercially at ATCC. All three CML cell lines are derived from patients in blast crisis with the particularity that the K562 cells are highly undifferentiated granulocytic cells derived from a terminal blast crisis while the MEG-01 cells are megakaryoblasts derived from a megakaryoblastic crisis and the KU812 cells are peripheral blood

myeloblasts. A control, the U-937 cell line has been added as non-CML monocytic cells. Growth conditions have been set up for all described cell lines.

### **Evaluate PTP expression levels on CML cell lines**

Expression of human TC-PTP and PTP1B was assessed in all cell lines by western blot analysis as shown in figure 1. Human PTP1B is highly expressed in all three CML cell lines and is absent from the U-937 control cell line (left panel). As with PTP1B, human TC-PTP is also highly expressed in all three CML cells lines and absent from the control (right panel). B-actin has been used as protein loading control. We were unable to assess the expression of PTP-PEST in the CML cell lines. We used several antibodies to this phosphatase but none of them were recognizing specifically the human form of PTP-PEST by western blot analysis. Generation of antibodies against the human form of PTP-PEST will have to be initiated.

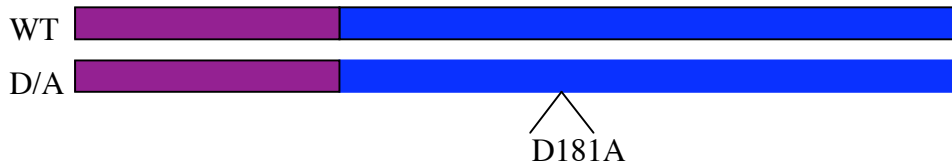


**Figure 1:** Immunoblots of hPTP-1B and hTCPTP from chronic myelogenous leukemia (CML) cell lysates and histiocytic lymphoma cell lysate as a control. Cell lines: 1. K562; 2. MEG01; 3. KU812; 4. U937.

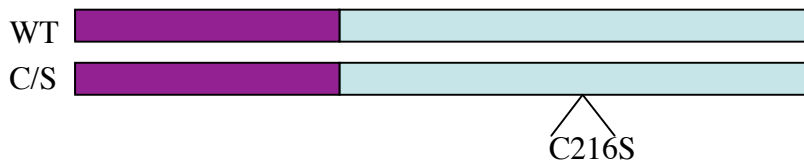
### **Generation of transgenic vectors**

The production of CML cell lines that overexpress TC-PTP and PTP1B or dominant negative mutants or these enzymes has been a slightly more time consuming task as we only had murine sequence and constructs available. After cloning human TC-PTP and PTP1B genes, the human PTP constructs were generated as shown in Figure 2. These dominant negative constructs are useful tools to identify potential substrates of TC-PTP and PTP1B and will identify signaling pathways regulated by TC-PTP and PTP1B in CML.

GFP-hPTP-1B (239+435 a.a.)



GFP-hTCPTP (239+387 a.a.)



**Figure 2.** Human PTP1B and TC-PTP constructs cloned in pc-DESt53. The mutation introduced to the wild type (WT) either (D/A or C/S) construct ablates the ability of the PTP to dephosphorylate target substrates but leaves substrate binding intact. Therefore, PTP-substrate complexes can be isolated by coimmunoprecipitation, and the substrates can subsequently be identified.

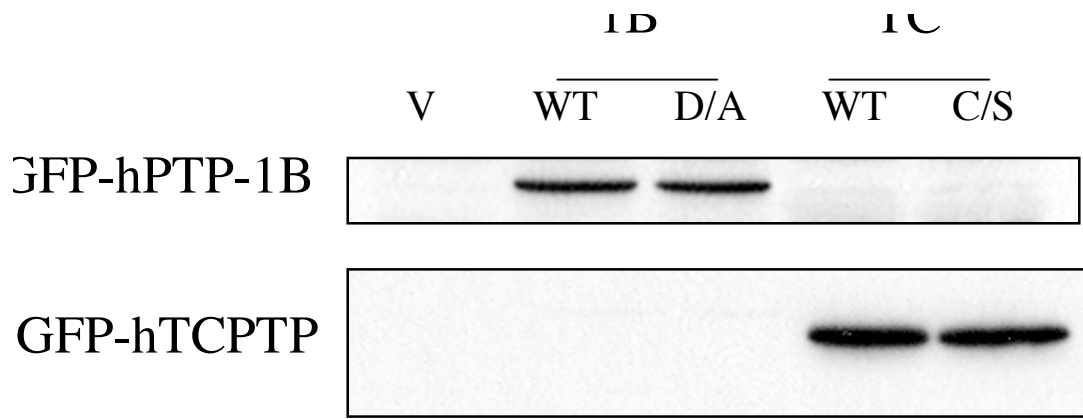
### **Identification of potential RNAi**

As shown in Figure 1, TC-PTP and PTP1B are expressed at very high levels in all selected CML cells lines. To look at the effect of downregulation of the expression of these PTPs we decided to design PTP-specific RNAi . Several RNAi have been selected from already published material and are thought to be effective as described in, Galic et al. 2005.

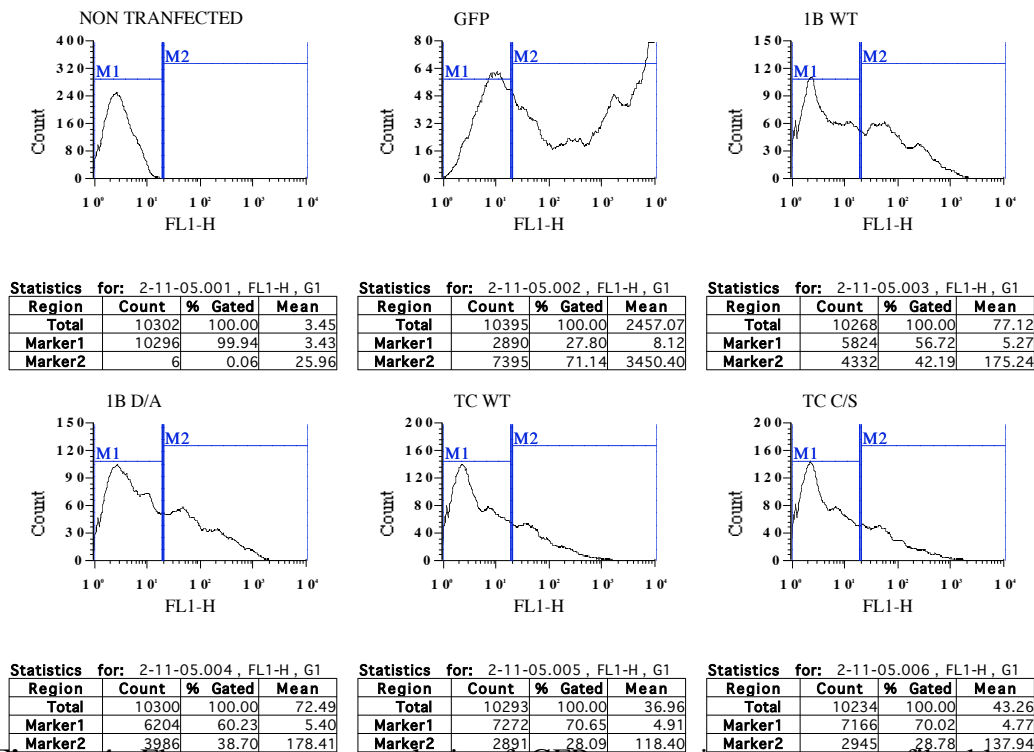
## **Task 2: Modulation of the PTPs in normal and leukemic cells**

### **Transfection of dominant negative constructs**

To assure that the WT and dominant negative constructs are able to be expressed by the cells, human PTP1B WT or D/A and TC-PTP WT or C/S were transfected in mouse fibroblast. Western blot analysis detecting the expression of either PTP1B or TC-PTP is shown in Figure 3. GFP was also detected by flow cytometry (Figure 4). We also transfected the vector alone (V), the GFP-hPTP1B (WT or D/A) and GFP-hTC-PTP (WT or C/S) constructs in K562 and U937 cells. We selected stable transfected cells with antibiotics (G418) but on the K562-V cells were able to proliferate. K562 and U937 cells are very sensitive to diluted growing condition. We are now in the process of redoing the antibiotic selection with a larger pool of transfected cells.



**Figure 3:** Immunoblots of hPTP1B and hTC-PTP. Mouse fibroblast cells have been transfected with the vector pcDNA-DEST53 (V), GFP-hPTP1B (WT or D/A) and GFP-hTC-PTP (WT or C/S)



**Figure 4:** Flow cytometry analysis of GFP expression on mouse fibroblast cells that have been non-transfected or transfected with the vector pcDNA-DEST53 (GFP), GFP-hPTP1B WT (IB WT) or GFP-hPTP1B D/A (1B D/A) and GFP-hTC-PTP WT (TC WT) or GFP-hTC-PTP C/S (TC C/S). The percentage of transfected cells GFP positive cells are identified by the M2 (Marker 2) region and are represented as percentage gated. The M1 (marker 1) represented GFP negative cells.

Task 3: Phenotypic evaluation of the ectopic expression of PTPs in leukemic cells and in animal models of CML.

To date we have obtained several mammalian expression constructs for the three PTPases. CML cell lines expressing at various levels these constructs are in process of being selected, in order to perform xenograft assay in nude mice. In the mean time, we developed and tested several FACS assays for various myeloid cells that will allow us to characterize the leukemia phenotype of the transfected cells. As an example please see Dube et al. 2005.

## Key Research Accomplishments

As key research accomplishment to date.

Task 1:

- Culture of leukemic cells
- Western blot analysis uncovering that as our hypothesis proposed, the two PTPs TC-PTP and PTP1b) are overexpressed in human leukemic cells.
- Mammalian expression vectors were generated and are functional.
- RNAi were identified from the literature and preliminary trestes were made for knocking down these two PTPases.

Task 2

- Dominant negative mutants were generated and transfected
- Macrophage analysis by FACS were done and tested

Task 3:

- Animal models are in progress
- FACS analysis of various myeloid populations was performed in mice.

## Reportable Outcomes

All Figures above have been performed and shown to be reproducibles.

The most interesting data is the ongoing analysis of the transfected cells in order to verifyt that using dominant negative mutants of these PTPases one may be able to restore the JAK signaling. If this is positive we would be able to report a first manuscript onthis work. At this stage the work is incomplete and cannot be reported as one single research finding.

## Conclusions

The research project undertaken with the CDMRP program has allowed us to confirm the presence of abherent amounts of the PTPs. These enzymes by their action on Jak signaling, when overexpressed have the potential to block interferon response. This is a major therapeutical approach in leukemic patients, hence the patients that develop resistance to this treatment have an outcome that is always severe and in many cases lethal. Our findings suggest that some of those interferon resistance phenomena may be caused by the overexpression of PTPs. Inhibition by small molecules or by antisense may be a potential new approach to restore IFN response in these patients allowing them to potentially be successfully treated by current regime. Our studies will thus continue in this direction in order to confirm the therapeutical value of inhibiting the PTPs in CML patients.

We are grateful for the support that CDMRP provided us and we trust that we will now be able to find alternative funding to complete our studies.

## References

- 1 Carlesso, N., D. A. Frank, et al. (1996). "Tyrosyl phosphorylation and DNA binding activity of signal transducers and activators of transcription (STAT) proteins in hematopoietic cell lines transformed by Bcr/Abl." J Exp Med **183**(3): 811-20.
- 2 Caspersson, T., G. Gahrton, et al. (1970). "Identification of the Philadelphia chromosome as a number 22 by quinacrine mustard fluorescence analysis." Exp Cell Res **63**(1): 238-40.
- 3 Dube N, Bourdeau A, Heinonen KM, Cheng A, Loy AL, Tremblay ML. Genetic ablation of protein tyrosine phosphatase 1B accelerates lymphomagenesis of p53-null mice through the regulation of B-cell development. *Cancer Res.* 2005 Nov 1;65(21):10088-95.
- 4 Galic S., et al. (2005) Coordinated regulation of insulin signaling by the protein tyrosine phosphatases PTP1b and TC-PTP. *Mol. Cell Biol.* 25:819-829.
- 5 Gu, F., N. Dube, et al. (2003). "Protein tyrosine phosphatase 1B attenuates growth hormone-mediated JAK2-STAT signaling." Mol Cell Biol **23**(11): 3753-62.
- 6 Haspel, R. L. and J. E. Darnell, Jr. (1999). "A nuclear protein tyrosine phosphatase is required for the inactivation of Stat1." Proc Natl Acad Sci U S A **96**(18): 10188-93.
- 7 Ilaria, R. L., Jr. and R. A. Van Etten (1996). "P210 and P190(BCR/ABL) induce the tyrosine phosphorylation and DNA binding activity of multiple specific STAT family members." J Biol Chem **271**(49): 31704-10.
- 8 Kaszubska, W., H. D. Falls, et al. (2002). "Protein tyrosine phosphatase 1B negatively regulates leptin signaling in a hypothalamic cell line." Mol Cell Endocrinol **195**(1-2): 109-18.
- 9 LaMontagne, K. R., Jr., A. J. Flint, et al. (1998). "Protein tyrosine phosphatase 1B antagonizes signalling by oncoprotein tyrosine kinase p210 bcr-abl in vivo." Mol Cell Biol **18**(5): 2965-75.
- 10 LaMontagne, K. R., Jr., G. Hannon, et al. (1998). "Protein tyrosine phosphatase PTP1B suppresses p210 bcr-abl-induced transformation of rat-1 fibroblasts and promotes differentiation of K562 cells." Proc Natl Acad Sci U S A **95**(24): 14094-9.
- 11 McBride, K. M., C. McDonald, et al. (2000). "Nuclear export signal located within the DNA-binding domain of the STAT1 transcription factor." Embo J **19**(22): 6196-206.
- 12 Myers, M. P., J. N. Andersen, et al. (2001). "TYK2 and JAK2 are substrates of protein-tyrosine phosphatase 1B." J Biol Chem **276**(51): 47771-4.
- 13 Rane, S. G. and E. P. Reddy (2002). "JAKs, STATs and Src kinases in hematopoiesis." Oncogene **21**(21): 3334-58.
- 14 Shtivelman, E., B. Lifshitz, et al. (1985). "Fused transcript of abl and bcr genes in chronic myelogenous leukaemia." Nature **315**(6020): 550-4.
- 15 Simoncic, P. D., A. Lee-Loy, et al. (2002). "The T cell protein tyrosine phosphatase is a negative regulator of janus family kinases 1 and 3." Curr Biol **12**(6): 446-53.
- 16 Steelman, L. S., S. C. Pohnert, et al. (2004). "JAK/STAT, Raf/MEK/ERK, PI3K/Akt and BCR-ABL in cell cycle progression and leukemogenesis." Leukemia **18**(2): 189-218.
- 17 ten Hoeve, J., M. de Jesus Ibarra-Sanchez, et al. (2002). "Identification of a nuclear Stat1 protein tyrosine phosphatase." Mol Cell Biol **22**(16): 5662-8.
- 18 Tough, I. M., W. M. Court Brown, et al. (1961). "Cytogenetic studies in chronic myeloid leukaemia and acute leukaemia associated with monogolism." Lancet **1**: 411-7.

**Supporting data:** Please see figures in the text.

**Appendices:**