NorCal Open Access Publications
Journal of Blood Transfusion and Hematopathology
Volume 1; Issue 1
Qazil S

Editorial

Wilms' Tumor Gene (WT1) Expression in Cytogenetically Distinct Subsets of AML

Sanjive Qazi1[1-5] and Fatih Uckun[2-5*]

[1]Bioinformatics Program, Gustavus Adolphus College, St. Peter, MN 56082, USA
[2]AresMIT Biomedical Computational Strategies (ABCS), Minneapolis, MN 55402, USA
[3]Ares Pharmaceuticals, LLC, St. Paul, MN 55110, USA
[4]Division of Hematology-Oncology, Department of Pediatrics USC KSOM, LA, CA 90027, USA
[5]Norris Comprehensive Cancer Center, University of Southern California Keck School of Medicine (USC KSOM), Los Angeles, CA 90027, USA

[*]**Corresponding author:** Fatih Uckun, AresMIT Biomedical Computational Strategies (ABCS), Minneapolis, MN 55402, USA, Tel: +1 6128106032; E-mail: fatih.uckun@aresmit.com

Received Date: 02 January, 2018; **Accepted Date:** 24 January, 2018; **Published Date:** 10 February, 2018

The Wilms' tumor gene (WT1) [1-3] is a tumor-suppressor gene associated with Wilms' tumor, a pediatric kidney cancer. The protein product of the WT1 gene is a zinc-finger transcription factor and a tumor-associated antigen. While WT1 is an intracellular protein, WT1-derived peptides are expressed on the surface of cancer cells in the context of HLA Class I antigens. As such they can be targeted by bispecific T-cell engagers (BiTEs), CAR-T cells and WT1-specific CTL that expand after WT1 peptide vaccine treatments [4-19].

WT1 is abundantly expressed in numerous hematological malignancies, including Acute Myeloid Leukemia (AML) [3,20-25]. WT1 expression may be induced by leukemia-associated oncogenes (like PML-RARA) as a physiologic, inhibitory response to oncogenic stress. In this case, subsequent inactivating mutations in WT1 would provide an additional growth advantage [20]. Oncomine database survey of WT1 mRNA expression in AML vs. non-leukemic healthy bone marrow samples showed 2-23-fold higher expression levels in AML samples with highly significant P-values for all studies deposited in the Oncomine database (Table 1) [26-29].

Cancer Tissue	P-value	T-Statistic	Fold change	Oncomine Reference	Normal (N)	Cancer (N)	Accession #	Pub
Acute Myeloid Leukemia	2.8×10^{-11}	18.5	23	Valk Leukemia	8	285	GSE1159	15-Apr-04
Acute Myeloid Leukemia	9.07×10^{-4}	3.9	11.5	Stegmaier Leukemia	6	9	GSE995	30-Jan-04
Acute Myeloid Leukemia	1.34×10^{-126}	31.5	3.1	Haferiach Leukemia	74	542	GSE13159	30-Sep-09
Acute Myeloid Leukemia	2.01×10^{-5}	5.5	3.1	Andersson Leukemia	5	18	GSE7186	15-Apr-07
Acute Myeloid Leukemia	2.44×10^{-51}	18.7	2	Haferiach Leukemia 2	58	257	GSe13164	30-Sep-09

Table 1: Oncomine Survey of WT1 mRNA Expression in AML vs Healthy Non-Leukemic Bone marrow samples.

We utilized an online data mining tool harboring 715 datasets with 86733 samples for cancer patients and cell lines to assess expression of WT1 across major cancer subtypes (https://www.oncomine.org/resource/main.html). We focused our analysis on mRNA expression comparing non-leukemic normal versus AML samples (5 independent comparisons, $\log_2$ median centered expression values for each data set) and filtered expression signals that were greater than 1.5-fold difference and p-value from two sample t-tests less than 0.001. WT1 expression was ranked according to Fold change. The table depicts the T-statistic, P-value, sample sizes for non-leukemic normal and AML samples for each study that were also referenced to the Gene Expression Omnibus identifier (GSE#). All 5 studies [26-29] in the Oncomine database exhibited significant increase in WT1 expression for AML samples (Fold change ranged from 2 to 23 relative to non-leukemic bone marrow samples).

We next interrogated the GSEA13159 database of gene expression data from the MILE Study (Microarray Innovations in Leukemia), that were generated utilizing the Affymetrix Human Genome U133 Plus 2.0 Array platform, for WT1 mRNA expression in cytogenetically distinct subsets of AML patients (N=542). The MILE study compared gene expression profiles for subsets of leukemia defined by gold standard laboratory techniques, and controlled for statistical batch effects from hybridizations measured at different clinics utilizing pre-processing methods including DQN3 normalization of the detected signals.

As detailed in Table 1, AML samples exhibited 4.9-9.7 fold higher expression levels of WT1 than normal bone marrow samples and the documented differences were highly statistically significant. Further, several of the WT1-regulated genes, such as EWSR1, WTAP, U2AF2, and PAWR were also expressed at significantly higher levels in AML samples [27]. As depicted in figure 1 and table 2, samples from AML patients with inv(16) (9.3-fold amplification, $P<10^{-16}$) or t(15;17) (9.7-fold amplification, $P<10^{-16}$) showed the highest WT1 expression levels.

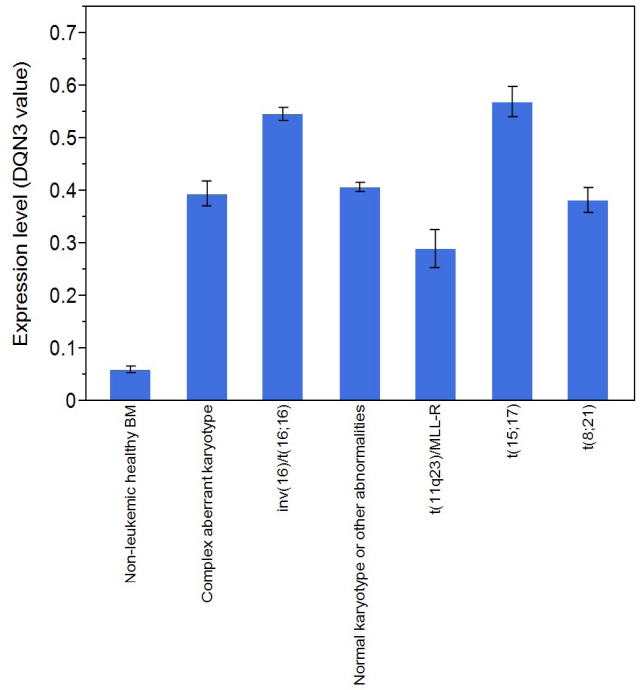

Figure 1: Increased levels of WT1 expression in AML subsets compared to normal samples from the MILE study. Gene expression profiling data utilizing the Affymetrix Human Genome U133 Plus 2.0 Array from the MILE Study were interrogated for WT1 expression in AML samples relative to normal non-leukemic healthy bone marrow samples. DQN3-normalized expression levels for the WT1 probeset, 206067_s_at in cytogenetically distinct AML subsets, including AML with t(8;21)(q22;q22.1) translocation, AML with inversion (inv)(16)(p13.1q22) or t(16;16)(p13.1;q22) translocation, AML with t(15;17)(q24.1;q21.2) translocation (APL with PML-RARA according to WHO classification), AML with 11q23 rearrangements involving the MLL gene (t(11q23)/MLL-R) including t(9;11)(p21.3;q23.3), AML with a complex aberrant karyotype, and AML with a normal karyotype or other abnormalities (e.g., t(1;22)(p13.3;q13.3), inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2), t(6;9)(p23;q34.1), are depicted using mean and standard error values presented in the bar chart. AML with inv (16) and AML with t(15;17) exhibited the highest expression levels for WT1. MLL-R, MLL gene rearranged. t, translocation. inv, inversion. BM, bone marrow.

AML subset	N (% of Total)	Fold Increase (vs Non-leukemic CON)	P-value (Dunnett's Test)
inv(16)/t(16;16)	28 (5%)	9.3	$<10^{-16}$
t(8;21)	40 (7%)	6.5	$<10^{-16}$
t(15;17)	37 (7%)	9.7	$<10^{-16}$
11q23/MLL-R	38(7%)	4.9	6×10^{-14}
Normal karyotype or other abnormality	351 (65%)	6.9	$<10^{-16}$
Complex aberrant karyotype	48 (9%)	6.7	$<10^{-16}$

Table 2: WT1 expression in cytogenetically distinct AML subtypes (N=542).

One way ANOVA was performed across cytogenetically distinct subsets of AML in the MILE study and the statistical significance for the observed differences (vs. non-leukemic normal control samples) was assessed using the Dunnett's post hoc tests. Mean Fold change and P-values following the Dunnet's post hoc tests are depicted in the table. Compared to normal samples, leukemic samples from each of the 6 AML subsets exhibited greater than 5-fold increase in WT1 expression levels for the WT1 probeset, 206067_s_at. CON: normal control.

In AML, the levels of WT1 gene expression correlate with prognosis: Patients with high-level expression have a lower likelihood to respond to induction chemotherapy and achieve complete remission [21]. A short 4-gene signature associated with high WT1 expression levels and the resultant 4-gene expression score were found to be predictive of adverse prognosis in AML [3]. A recent meta-analysis confirmed the significantly poor prognostic impact on OS and DFS of patients with AML in total population and some specific subgroups [23]. Therefore, the level of WT1 expression can add prognostic information in AML risk stratification [24]. High WT1 expression has been shown to be an early predictor of relapse in patients with acute promyelocytic leukemia in remission [21]. Candoni et al., analyzed the outcome of allogeneic hematopoietic stem cell transplantation in a population of FLT3-positive AML patients according to molecular Minimal Residual Disease (MRD) at the pretransplant workup, assessed by the quantitative expression evaluation of the WT1 gene [25]. Patients with undetectable WT1 mRNA levels in their pre-transplant remission bone marrow (i.e., a

WT1-negative complete remission) before had a very good outcome with low relapse rate post-transplant; conversely, patients whose bone marrows tested positive for WT1 mRNA (i.e., WT1-positive complete remission) consistent with the presence of a molecular MRD as well as refractory/relapsed patients had a poor outcome [25]. The authors concluded that the WT1 MRD stratification in FLT3-positive AML may serve as a valuable tool to identify patients who are at a high risk for relapse and might benefit from post-transplant intervention with FLT3 inhibitors [25]. Dulery et al., reported the usefulness of peripheral blood MRDWT1 monitoring in identifying very high-risk patients, who could benefit from an early preemptive treatment, and those who do not need such an intervention [22].

Several WT1-specific targeted cancer immunotherapy modalities, including therapeutic vaccines, WT1-specific CTL or CAR-T cell platforms, are currently being evaluated in clinical trials for multiple therapeutic indications, including AML [4-19]. Scheinberg et al., have been pursuing WT1 peptide vaccines (viz., galinpepimut-S) and recently reported novel synthetic WT1 peptide designs for more effective immunization/CTL generation using advanced computer modeling tools [6]. Further, Advaxis and Sellas (a subsidiary of Galena Biopharma - (NASDAQ:GALE) completed a licensing agreement for using a WT1-targeted heteroclitic peptide antigen mixture (galinpepimut-S/GPS licensed from Memorial Sloan Kettering Cancer Center) in the context of the Advaxis' Lm-based antigen delivery technology aimed at reducing immune tolerance in the tumor microenvironment. SELLAS has been granted orphan drug designation from the FDA and the European Medicines Agency (EMA) and been given FDA fast track status.

Recently, a T-Cell Receptor (TCR) that specifically reacts with the WT1 peptide in the context of HLA-A*24:02 has been identified. This WT1-specific TCR-gene was transduced to autologous T-cells using a retroviral vector encoding small interfering RNAs for endogenous TCR genes to generate WT1-specific TCR-T cells capable of executing an immune reaction to WT1-expressing AML cells. The early results of the first-in-human Phase 1 clinical trial (www.umin.ac.jp as #UMIN000011519) of this WT1 TCR transduced T-cell therapy (also known as WT1 antigen-specific RetroNectin TCR gene therapy - Takara Bio) in therapy-refractory AML patients have been reported [18]. Likewise, ATA520 (Atara Biotherapeutics) is a Cytotoxic T Lymphocyte (CTL) product candidate against WT1 (i.e., WT1-CTL) that is being studied in multiple clinical trials for its safety and activity as a targeted biotherapeutic agent against WT1-positive hematologic malignancies. Shah et al., reported the safety and feasibility of a WT1 peptide-loaded donor-derived Dendritic Cell (DC) vaccine given with donor lymphocyte infusions designed to enhance and direct the graft-versus-leukemia effect [4]. Preliminary results indicate the DC-based vaccination is safe and feasible after allogeneic HCT, and suggest that this strategy could potentially be used to sensitize the repopulated allogeneic-donor immune system to $WT1^+$ leukemia cells.

Current Challenges and Future Directions

The landscape of available treatment strategies for AML has changed dramatically with the recent FDA approval several targeted therapeutic agents with clinical activity in AML [30]. Recent vaccination approaches to target WT1 have been hampered by poor in vivo immune potency, likely due to the conserved self-antigen nature of WT1. That is why some groups are exploring the use of synthetic consensus DNA vaccines for breaking tolerance to this germline antigen [19]. Even in AML with very high level WT1 expression, the T-cell responses and prolonged remissions reported in AML patients treated with this approach have generally been limited to the setting of MRD (i.e., not observed in the setting of overt disease). Engineered CAR-T cells or WT1-CTLs are more likely to overcome these inherent challenges of poor immunogenicity of WT1.

References

1. Kim MK, McGarry TJ, Broind PO, Flatow JM, Goldend AAJ, et al. (2009) An integrated genome screen identifies the Wnt signaling pathway as a major target of WT1. PNAS 106: 11154-11159.

2. Ayatollahi H, Sadeghian MH, Naderi M, Jafarian AH, Shams SF, et al. (2017) Quantitative assessment of Wilms tumor 1 expression by realtime quantitative polymerase chain reaction in patients with acute myeloblastic leukemia J Res Med Sci 22: 54.

3. Niavarani A, Herold T, Reyal Y, Sauerland MC, Buchner T, et al. (2016) A 4-gene expression score associated with high levels of Wilms Tumor-1 (WT1) expression is an adverse prognostic factor in acute myeloid leukaemia. British Journal of Haematology 172: 401-411.

4. Shah NN, Loeb DM, Khuu H, Stroncek D, Ariyo T, et al. (2016) Induction of Immune Response after Allogeneic Wilms' Tumor 1 Dendritic Cell Vaccination and Donor Lymphocyte Infusion in Patients with Hematologic Malignancies and Post-Transplantation Relapse. Biol Blood Marrow Transplant 22: 2149-2154.

5. Zauderer MG, Tsao AS, Dao T, Panageas K, Lai WV, et al. (2017) A Randomized Phase II Trial of Adjuvant Galinpepimut-S, WT-1 Analogue Peptide Vaccine, After Multimodality Therapy for Patients with Malignant Pleural Mesothelioma. Clin Cancer Res 23: 7483-7489.

6. Dao T, Korontsvit T, Zakhaleva V, Jarvis C, Mondello P, et al. (2016) An immunogenic WT1-derived peptide that induces T cell response in the context of HLA-A*02:01 and HLA-A*24:02 molecules. Oncoimmunology 6: e1252895.

7. Rafiq S, Purdon TJ, Daniyan AF, Koneru M, Dao T, et al. (2017) Optimized T-cell receptor-mimic chimeric antigen receptor T cells directed toward the intracellular Wilms Tumor 1 antigen. Leukemia 31: 1788-1797.

8. Ataie N, Xiang J, Cheng N, Brea EJ, Lu W, et al. (2016) Structure of a TCR-Mimic Antibody with Target Predicts Pharmacogenetics. J Mol Biol 428: 194-205.

9. Dao T, Pankov D, Scott A, Korontsvit T, Zakhaleva V, et al. (2015) Therapeutic bispecific T-cell engager antibody targeting the intracellular oncoprotein WT1. Nat Biotechnol 33: 1079-1086.

10. Veomett N, Dao T, Liu H, Xiang J, Pankov D, et al. (2014) Therapeutic efficacy of an Fc-enhanced TCR-like antibody to the intracellular WT1 oncoprotein. Clin Cancer Res 20: 4036-4046.

11. Dao T, Liu C, Scheinberg DA (2013) Approaching untargetable tumor-associated antigens with antibodies. Oncoimmunology 2: e24678.

12. Dao T, Yan S, Veomett N, Pankov D, Zhou L, et al. (2013) Targeting the intracellular WT1 oncogene product with a therapeutic human antibody. Sci Transl Med 5: 176ra33.

13. O'Reilly RJ, Dao T, Koehne G, Scheinberg D, Doubrovina E (2010) Adoptive transfer of unselected or leukemia-reactive T-cells in the treatment of relapse following allogeneic hematopoietic cell transplantation. Semin Immunol 22: 162-172.

14. Krug LM, Dao T, Brown AB, Maslak P, Travis W, et al. (2010) WT1 peptide vaccinations induce CD4 and CD8 T cell immune responses in patients with mesothelioma and non-small cell lung cancer. Cancer Immunol Immunother 59: 1467-1479.

15. Maslak PG, Dao T, Krug LM, Chanel S, Korontsvit T, et al. (2010) Vaccination with synthetic analog peptides derived from WT1 oncoprotein induces T-cell responses in patients with complete remission from acute myeloid leukemia. Blood 116: 171-179.

16. Pinilla-Ibarz J, May RJ, Korontsvit T, Gomez M, Kappel B, et al. (2006) Improved human T-cell responses against synthetic HLA-0201 analog peptides derived from the WT1 oncoprotein. Leukemia 20: 2025-2033.

17. Gomez-Nunez M, Pinilla-Ibarz J, Dao T, May RJ, Pao M, et al. (2006) Peptide binding motif predictive algorithms correspond with experimental binding of leukemia vaccine candidate peptides to HLA-A*0201 molecules. Leuk Res 30: 1293-1298.

18. Tawara I, Kageyama S, Miyahara Y, Fujiwara H, Nishida T, et al. (2017) Safety and persistence of WT1-specific T-cell receptor gene-transduced lymphocytes in patients with AML and MDS. Blood 130: 1985-1994.

19. Walters JN, Ferraro B, Duperret EK, Kraynyak KA, Chu J, et al. (2017) A Novel DNA Vaccine Platform Enhances Neo-antigen-like T Cell Responses against WT1 to Break Tolerance and Induce Anti-tumor Immunity. Mol Ther 25: 976-988.

20. Christopher M, Menssen A, Gang M, Upadhyaya A, Wartman LD, et al. (2017) Expression of the Tumor Suppressor WT1 is Induced By PML-Rara in Acute Promyelocytic Leukemia. Blood 130: 2508.

21. Yoon JH, Kim HJ, Kwak DH, Park SS, Jeon YW, et al. (2017) High WT1 expression is an early predictor for relapse in patients with negative PML-RARa after anthracycin-based chemotherapy: a single-center cohort study. J Hematol Oncol 10: 30.

22. Duléry R, Nibourel O, Gauthier J, Elsermans V, Behal H, et al. (2017) Impact of Wilms' tumor 1 expression on outcome of patients undergoing allogeneic stem cell transplantation for AML. Bone Marrow Transplant 52: 539-543.

23. Yi-Ning Y, Xiao-rui W, Chu-xian Z, Chun W, You-wen Q (2015) Prognostic significance of diagnosed WT1 level in acute myeloid leukemia: a meta-analysis. Ann Hematol 94: 929-938.

24. Hecht A, Nolte F, Nowak D, Nowak V, Reinwald M, et al. (2015) Prognostic importance of expression of the Wilms' tumor 1 gene in newly diagnosed acute promyelocytic leukemia. Leuk Lymphoma 56: 2289-2295.

25. Candoni A, De Marchi F, Zanini F, Zannier ME, Simeone E, et al. (2017) Predictive value of pretransplantation molecular minimal residual disease assessment by WT1 gene expression in FLT3-positive acute myeloid leukemia. Exp Hematol 49: 25-33.

26. Valk PJ, Verhaak RG, Beijen MA, Erpelinck CA, Barjesteh van Waalwijk, et al. (2004) Prognostically useful gene-expression profiles in acute myeloid leukemia. N Engl J Med 350: 1617-1628.

27. Stegmaier K, Ross KN, Colavito SA, O'Malley S, Stockwell BR, et al. (2004) Gene Expression-based High-Throughput Screening (GE-HTS) and application to leukemia differentiation. Nat Genet 36: 257-263.

28. Haferlach T, Kohlmann A, Wieczorek L, Basso G, Kronnie GT, et al. (2010) Clinical utility of microarray-based gene expression profiling in the diagnosis and subclassification of leukemia: report from the International Microarray Innovations in Leukemia Study Group. J Clin Oncol 28: 2529-2537.

29. Andersson A, Ritz C, Lindgren D, Edén P, Lassen C, et al. (2007) Microarray-based classification of a consecutive series of 121 childhood acute leukemias: prediction of leukemic and genetic subtype as well as of minimal residual disease status. Leukemia 21: 1198-203.

30. Menghrajani K, Tallman MS (2017) New therapeutic strategies for high-risk acute myeloid leukemia. Curr Opin Hematol 25: 90-94.