Review of the results of WT1 peptide vaccination strategies for myelodysplastic syndromes and acute myeloid leukemia from nine different studies

Antonio Di Stasi1*, Antonio M. Jimenez2, Kentaro Minagawa1, Mustafa Al-Obaidi1 and Katayoun Rezvani3*

1 Stem Cell Transplantation and Cell Therapy Unit, The University of Alabama at Birmingham, Birmingham, AL, USA
2 Stem Cell Transplantation and Cell Therapy Unit, Rush University Medical Center, Chicago, IL, USA
3 Stem Cell Transplantation and Cell Therapy Unit, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

We performed a systematic review of data from nine clinical trials of WT1 peptide vaccination in patients with myelodysplastic syndromes and/or acute myeloid leukemia (MDS/AML), published between 2004 and 2012. A total of 51 patients were eligible for analysis. Vaccination with WT1 peptides proved safe and feasible in patients with MDS/AML, in studies from different institutions. Additionally, clinical responses and clinical benefit were observed, with some patients achieving and maintaining remission long-term (more than 8 years). A significant correlation between induction of WT1-specific T cells and normalization/reduction of WT1 mRNA levels and progression-free survival was noted in a number of studies. However, larger studies are warranted to confirm these results. Interestingly, the majority of trials reported the presence of WT1-specific T cells with limited or absent functionality prior to vaccination, which increased in frequency and function after vaccination. In conclusion, WT1 peptide vaccination strategies were safe in this heterogeneous group of patients with MDS/AML. Larger and more homogeneous studies or randomized clinical trials are needed to quantify the contribution of WT1 peptide vaccines to clinical responses and long-term survival.

Keywords: WT1, peptide vaccine, MDS/AML, TAA, active immunotherapy
induce anti-tumor activity without inhibiting engraftment of normal CD34+ hematopoietic progenitor cells (28). The selectivity of WT1-specific human T cells as effectors against WT1 expressing targets has also been shown in vitro (29) and several epitopes, including helper T cell epitopes, have entered clinical trials (30).

Most TAAs are aberrantly expressed self-proteins, and T cells directed against these antigens typically express low-affinity T cell receptors as a consequence of the negative selection in the thymus. In contrast, when stimulated with low doses of foreign antigens in combination with noxious substances (adjuvants), the immune system is activated, leading to the generation of effector and memory T cells (31).

The success of a particular peptide vaccine to elicit an immune response is influenced by many parameters, including the presence of helper T cell epitopes, processing and presentation by professional antigen presenting cells (APCs), bio-distribution, influence of adjuvants, peptide length, peptide affinity, and mode of administration (Table 1).

This review, we will summarize the immunologic and clinical results of WT1 peptide vaccination approaches in patients with myelodysplastic syndromes and/or acute myeloid leukemia (MDS/AML) (31).

**CLINICAL STUDIES OF WT1 PEPTIDE VACCINES IN MDS/AML**

A detailed report of the clinical studies reviewed in this article is presented in Table 2 (5–14).

In order to analyze survival outcomes after vaccination, we combined the results from seven reports published between 2004 and 2012 (5, 7, 9, 10, 12–14). Unduplicated observations were available for 55 out of 67 patients with MDS/AML: 4 patients were not evaluable for response, and therefore the final number of evaluable patients in our analysis was 51. A summary of the patients and their responses is detailed in Table 2. The majority of treated patients received also granulocyte monocyte colony stimulating factor injections, and the majority were vaccinated against an epitope recognized in the context of human leukocyte antigen HLA-A02-01; however, some studies employed HLA-A24-02 (36–39), and in one study peptide recognized in the context of HLA-A02-01 were administered together with peptide recognized in the context of HLA-DRB1 (41).

We first evaluated if vaccination with WT1 peptide was reported to induce expansion of WT1-specific T cells. By analyzing the results published in four trials (23 patients) where WT1-specific T cells were estimated by tetramer analysis or ELIspot assay without ex vivo expansion (6, 8, 11, 14), we were able to estimate that WT1 vaccination resulted in an overall median fold expansion in WT1-specific T cell frequencies of 2.4, as compared with baseline. The tetramer positive T cells increased from a median of 0.14% (range 0–0.98%) pre vaccination to 0.41% (range 0–6.6%) post-vaccination. Rezvani et al. (11) reported that the absolute number of CD3+CD8+ WT1 tumor T cells increased from a median value of 95 per mL pre vaccination, (range 20–423 cells/mL), to 398 per mL after vaccination (range 98–4570 cells/mL).

Keilholz et al. (7) reported a significant increase in the median frequency of WT1 tetramer positive T cells in the bone marrow from 0.18% (week 0) to 0.41%, at week 18 after vaccination (P = 0.04). In the peripheral blood, WT1 tetramer positive cells were present at 0.12% at baseline, increasing to 0.28% at week 10, and persisting at stable levels (0.25%) at week 18, although these values did not reach statistically significance. The authors reported that only patients with low blast count in the bone marrow at baseline (<40% blasts, n = 9) had a statistically significant expansion in the peripheral blood of WT1 tetramer positive T cells after vaccination, as compared with patients with a high blast count (>50% blasts, n = 9); the median frequencies at week 0, 10, and 18 were 0.11, 0.30, and 0.46% (P < 0.01) vs. 0.12, 0.27, and 0.23%, in the two groups, respectively. Interestingly, four patients in the low blast group had a functional WT1 T cell response [gamma-interferon (IFN)-gamma production] compared with only one patient in the high blast group. In all the evaluable patients from these studies, although WT1-specific T cells were present in vivo at low frequencies prior to vaccination, the functional response after WT1 peptide stimulation measured as IFN-gamma production was limited or absent, increasing only after vaccination.

In the study by Maslak et al. (13), a combination of WT1 peptides comprising of one short peptide with a mutated R126Y (heteroclitic) epitope (to elicit CD3+CD8+ T cells), two long peptides (to elicit CD3+CD4+ T cells), and one long peptide with the heteroclitic sequence (to elicit both CD3+CD4+ and CD3+CD8+ T cells) were tested. Ex vivo experiments with CD3+CD4+ T cells isolated from vaccinated patients showed that WT1-specific functional responses were stronger against the CD4 epitopes, although one patient showed a strong response toward the CD3+CD8+ heteroclitic WT126–134 peptide. Interestingly, long peptides elicited the strongest immunological responses in vitro, and an IFN-gamma ELIspot assay performed after two rounds of in vitro stimulation showed that both native and heteroclitic peptides could elicit strong functional responses against WT1. Although the HLA-DR heteroclitic peptide was more efficient than its native counterpart, both elicited responses against both

---

**Table 1 | Strategies to improve the efficacy of anti-tumor vaccination.**

| Improve response | 1) Presence of appropriate cytokines  
2) Use of Thelper epitopes or DC agonists (TNF, TLR, and PADRE)  
3) Slow release vaccines  
4) Draining to local activated lymph nodes  
5) Avoid continuous or repeated administration, which can induce Tregulatory cells  
6) Peptide elongation |
| Prevent systemic spread | 1) Attachment of lipid tails to peptides  
2) Linking APC activating compounds and antigen  
3) Adoption of linkers between cytotoxic and helper sequences |
| Reduce toxicity | 1) Avoid quick and widespread bio-distribution (cytokine storm)  
2) Avoid high doses or repeated administration  
3) Identify bio-markers to predict and monitor toxicity |

TNF: tumor necrosis factor; TLR: toll like receptor; PADRE, T cell Pan DR epitope; APC, antigen presenting cells.

---

**Table 2**

| Patient Characteristics | N (%) | Median Response | 95% CI |
|------------------------|-------|----------------|--------|
| Low blast count        | 40    | 0.28%          | 0.12–0.41% |
| High blast count       | 13    | 0.12%          | 0.03–0.27% |

---

**February 2015 | Volume 6 | Article 36 | 2**
Table 2 | Summary of reviewed clinical trials

| Diagnosis (N) | Disease status/ [previous tx] | Epitope | Vax# (range); [adjuvant] | (N) Toxicity grade III-IV | Anti WT1 responses | Clinical responses | [Follow-up]/ [response duration] | Reference |
|--------------|-------------------------------|---------|--------------------------|--------------------------|---------------------|--------------------|-----------------------|-----------|
| AML (1)      | 1PR [chemo]                   | WT1<sub>126-134</sub> | 15; [KLH]                | None                     | Yes                 | Yes                | (1) Morphological/ molecular CR | [46 weeks]/[30 weeks] | (6)       |
| AML (17)     | 13 PD                         | WT1<sub>126-134</sub> | 11(4–27); [KLH]          | None                     | N/A                 | Yes                | (1) CR, (13) SD, (4) PD, (1) Major neutrophil response | [NA] [CR: 16 months; FFS SD 155D (101–571)] | (7)       |
| MDS (2)      | 6 PR, 2 EB [chemo]            |         |                          |                          |                     |                    |                                      |           |           |
| AML (12) MDS | other (12)                    |         | Natural WT1<sub>235-243</sub> vs. modified | 3; [mlSA51]              | None                 | Yes                | (5) Molecular CR (2) PR, (1) SD, (2) PD, (4) NE | [NA]      |           |
| AML (3)      | 3 MRD [chemo]                 | Natural WT1<sub>235-243</sub> vs. modified | Several; [mlSA51]        | None                     | Yes                 | Yes                | (3) CR for >8 years | [90 months (90–94)] [NA] | (9)       |
| AML (5) MDS  | (2) other (1)                 | PR1<sub>169-177</sub> and WT1<sub>126-134</sub> | 6; [mlSA51]              | None                     | Yes                 | Yes                | (3) CCR, (2) SD, (2) relapse | [NA] [SD: 180D (105–523)] | (11)      |
| AML (6)      | 6 CR [5 chemo, 1 allo-HSCT]   | PR1<sub>169-177</sub> and WT1<sub>126-134</sub> | 6; [mlSA51]              | None                     | N/A                 | Yes                | (2) CCR, (1) SD, (1) PD, (4) relapse | [NA] [SD: 832D, CCR: 683D (587–779), TTR: 112D (14–352)] | (10)      |
| MDS (2)      | 1 RA, 1 RARS [EPO/GCSF]       |         |                          |                          |                     |                    |                                      |           |           |
| AML (1)      | 1 AD [chemo]                  | WT1<sub>235-243</sub> | 20; [mlSA51]             | None                     | N/A                 | Yes                | (1) Morphological CR, (1) molecular CR | [NA] [CR > 3 years] | (12)      |
| MDS (1)      | 1 MRD [NA]                    |         |                          |                          |                     |                    |                                      |           |           |
| AML (9)      | 9 MRD [chemo]                 | WT1<sub>126-134</sub> and WT1<sub>427-449/333/352/122-140</sub> | 9 (6–12); [mlSA51]       | None                     | Yes                 | Yes                | (5) CCR, (4) relapse | [NA] [DFS 31 months (10–121), mPFS not reached] | (13)      |
| AML (4)      | 3 AD [NA]                     | WT1<sub>126-134</sub> and PR3<sub>169-177</sub> with PADRE/MUC1 helper epitope | 6; [CPG7909/mlSA51]      | None                     | Yes                 | Yes                | (2) SD, (2) PD | [84D] [NA] | (5)       |

N, number; tx, treatment; vax, vaccine; WT1, Wilms’ tumor-1; AML, acute myeloid leukemia; PR, partial response; KLH, keyhole limpet hemocyanin; chemo, chemotherapy; (C)CR, (continuous) complete remission; MDS, myelodysplastic syndromes; PD, progressive disease; EB, excess blasts; NA, not available; IDDFS, (disease) progression-free survival; SD, stable disease; MRO, molecular residual disease; mlSA51, montanide ISA51; RA/RIS, refractory anemia (ringed sideroblasts); EPO, erythropoietin; GSCF, granulocyte colony stimulating factor; allo-HSCT, allogeneic hematopoietic stem cell transplantation; TTR, time to relapse; AD, active disease; AT<sup>+</sup>, mutated amino acid R126Y; PR3, proteinase-3; PADRE; T cell Pan DR epitope; MUC1, Mucin-1; CPG 7909, immunostimulatory toll-like receptor 9 (TLR9) agonist oligodeoxynucleotide.
the HLA-A02-01 and the HLA-DRB1 epitopes, indicating efficient processing and presentation of the HLA-A02-01 epitope embedded within the long peptide to CD8+ T cells.

Clinical responses and clinical benefit were observed in these studies, as reported in detail in Table 2, with some patients achieving and maintaining remission long-term (more than 8 years) (9). Of note, one patient had a complete response after the percentage of bone marrow blasts had reached 30% (7).

We also assessed whether correlation between WT1 responses and prognosis was reported in any of these studies. Some of the reviewed studies found a significant correlation between the detection of WT1-specific T cells and normalization/reduction of WT1 mRNA level [P < 0.01 (7, 11); P = 0.0397 (8)], whereas the loss of WT1-specific T cells was associated with reappearance of the WT1 transcript (11). A significant correlation was also reported between WT1 mRNA level and progression-free survival (P = 01), in one study (7).

Interestingly, in one study relapse was associated with the disappearance of T cell receptor clone restricted for Vbeta11 chain from the bone marrow (32), and a bias toward Vbeta11 usage of the WT1-specific T cells was further observed in four patients (33). In one patient, down-regulation of WT1 mRNA and loss of WT1 expression was observed at the moment of leukemia progression. However, additional immune-evasion mechanisms, such as WT1 mutation or loss of HLA expression on the surface of leukemic cells were not observed (34). Addressing other possible mechanisms resulting in loss of response to vaccination, Rezvani et al. (10) reported that repeated vaccinations eventually led to selective deletion of high avidity PR1- and WT1-specific CD3+ CD8+ T cells and was not associated with significant reduction in WT1 expression.

Additional boosting failed to increase vaccine-induced WT1+CD8+ T cell frequencies further and in all patients the response was lost before the sixth vaccine dose. Furthermore, the authors of another report suggested a negative impact of using the immunostimulatory toll like receptor 9 (TLR9) agonist oligodeoxynucleotide (CPG7909), and Montanide ISA51 (mISA51) as adjuvants for the vaccination (5).

Finally, in all the analyzed studies, vaccination with WT1 was found to be safe and well tolerated, with only 8% of patients (7 out of 88 total patients with any diagnosis) experiencing grade III-IV toxicity.

**CONCLUSION**

Around 50% of patients undergoing allogeneic HSCT for MDS/AML experience long-term disease-free survival (2, 3), unfortunately, a significant proportion of patients will succumb to disease relapse (2). Alternative strategies are therefore urgently needed to improve outcomes, while also lowering treatment related mortalities and morbidities. The encouraging results to date from immunotherapeutic approaches, such as vaccination strategies, suggest that this option may offer a promising strategy to reduce the risk of disease relapse.

From the reports analyzed in our review, it is evident that vaccination with WT1 epitopes was safe, feasible, and potentially able to mediate sustained immune responses in patient with MDS/AML. Although these preliminary findings are encouraging, limitations of this review include the low number of patients in some of the analyzed clinical trials, and a heterogeneous group of patients with two different diseases diagnosis.

Although antigen-specific T cells for example against WT1 (35) and PR1 (36) are present in the blood of healthy donors and transferred to the patient after allogeneic HSCT or donor lymphocyte infusion, their persistence and expansion are transient, which may be explained by activation-induced apoptosis after exposure to high antigenic burden (37), or terminally differentiated effector memory phenotype (38). Therefore, vaccination approaches can potentially enhance anti-TAA immune responses. However, a comprehensive understanding of the mechanisms underlying a successful vaccine-induced immune response and of the factors predictive of response would allow the design of optimal immunotherapeutic strategies for the treatment of patients with MDS/AML.

Administration of large or repeated doses of foreign antigens in order to enhance effectiveness of the vaccine proved not beneficial in our experience (10), as it led to induction of immune tolerance, potentially via T cell deletion, anergy, or expansion of antigen-specific regulatory T cells (31).

An alternative approach to counteract immune-evasion mechanisms, such as down-regulation of TAA expression, would be to combine different epitopes of the antigen of interest. Two reports summarized here, including one from our own group, explored the feasibility of vaccinating patients with epitopes derived from two different TAAs, however larger or randomized clinical trials are needed to demonstrate the superiority of this approach (5, 10).

Two strategies to help circumvent the need for T-helper cells with resulting more sustained anti-cancer T cell immunity have been investigated with success in murine models (39, 40): (i) the adoption of synthetic long-sequence peptides, and (ii) the use of adjuvants to stimulate APCs.

Synthetic long-sequence peptides are preferentially processed by professional APCs in the lymph node draining area, circumventing some of the tolerance mechanisms. In the study of Maslak et al. (13), long peptides with capacity to elicit both a CD3+CD8+ and a CD3+CD4+ T cell response, resulted in stronger immunological responses in vitro, but whether this strategy would prove effective in vivo is yet to be established.

Although agonistic anti-CD40 antibodies induced maturation of APCs preventing tolerance induction and circumventing the need for CD4+ T cell help in the early phase of T cell response, it did not prevent the long-term induction of tolerance, likely because once the anti-CD40 antibody had been cleared, peptides were presented to CD8+ T cells by tolerogenic APCs (41). One possible strategy to sustain antigen exposure with APCs in the draining lymph node would be to combine the peptide with lipid tails (31), and this approach has been investigated with encouraging results using FDA approved biodegradable poly(lactico-co-glycolic acid) microparticles, which shuttle antigens to the lymph nodes (42). To note, the replacement of mineral oils with novel delivery systems or the direct injection of peptides into lymph nodes (43) would also help in overcoming the long-term side effect of granuloma formation at the injection site observed with mISA51 (31).

Since persistence of antigen-specific T cells is required for successful immunotherapy, an optimal cytokine milieu (44, 45)
All the authors contributed to conception, acquisition, and analysis of data, participated in the manuscript draft preparation, revision and approved, and revised the final version.

**AUTHOR CONTRIBUTIONS**

All the authors contributed to conception, acquisition, and analysis of data, participated in the manuscript draft preparation, revision and approved, and revised the final version.

**REFERENCES**

1. Copelan EA. Hematopoietic stem-cell transplantation. *N Engl J Med* (2006) 354:1813–26. doi:10.1056/NEJMra052638
2. Cornelissen JJ, Gratwohl A, Schlenk RF, Sierra J, Bornhauser M, Juliusson G, et al. The European LeukemiaNet AML working party consensus statement on allogeneic HSCT for patients with AML in remission: an integrated-risk adapted approach. *J Natl Cancer Inst* (2012) 104:259–70. doi:10.1093/jnci/djs567
3. Zittoun RA, Mandelli F, Willemze R, de Witte T, Labar B, Rosti V, et al. Autologous or allogeneic bone marrow transplantation compared with intensive chemotherapy in acute myelogenous leukemia. European organization for research and treatment of cancer (EORTC) and the group italiano malattie ematologiche maligne Dell'adulto (GIMEMA) leukemia cooperative groups. *N Engl J Med* (1995) 332:217–23.
4. Sekeres MA. The euphoria of hypomethylating agents in MDS and AML: is it justified? Best Pract Res Clin Haematol (2013) 26:275–8. doi:10.1016/j.beha.2013.10.001
5. Kuhb J, de Boer K, Wagner E, Wottral M, Antunes E, Wicentz RD, et al. Pitfalls of vaccinations with WT1-, Proteinase 3- and MUC1-derived peptides in combination with Montanide ISA51 and Cpg7909. *Cancer Immunol Immunother* (2011) 60:161–71. doi:10.1007/s00262-010-0929-7
6. Mailander V, Scheibenbogen C, Thiel E, Letsch A, Blau IW, Keilholz U. Quantitative and qualitative changes of WT1 peptide vaccination following allogeneic stem cell transplantation in pediatric leukemic patients with high risk for relapse: successful maintenance of durable remission. *Leukemia* (2012) 26:530–2. doi:10.1038/leu.2011.226
7. Schmitt M, Schmitt A, Bojowski MT, Chen J, Giannopoulos K, Fei F, et al. Randomized clinical trials are needed to quantify the contribution of WT1 peptide vaccines to clinical responses and disease-free survival. *J Natl Cancer Inst* (2010) 101:1387–90. doi:10.1093/jnci/djp269
8. Oka Y, Tsuibo A, Oka K, Kyo T, Nakamura H, Hi, et al. Induction of WT1 (Wilms' tumor gene)-specific cytotoxic T lymphocytes by WT1 peptide vaccine and the resultant cancer regression. *Proc Natl Acad Sci U S A* (2004) 101:13885–90. doi:10.1073/pnas.0405884101
9. Tsuibo A, Oka K, Kyo T, Katayama Y, Elissieva OA, Kawakami M, et al. Long-term WT1 peptide vaccination for patients with acute myeloid leukemia with minimal residual disease. *Leukemia* (2012) 26:1410–3. doi:10.1038/leu.2011.343
10. Rezvani K, Yong AS, Mielke S, Jafarpour B, Savani BN, Le Q, et al. Repeated PRI and WT1 peptide vaccination in Montanide-adjunctive fails to induce sustained high-avidity, epitope-specific CD8+ T cells in myeloid malignancies. *Haematologica* (2011) 96:432–40. doi:10.3324/haematol.2010.031674
11. Rezvani K, Yong AS, Mielke S, Savani BN, Musse L, Superata J, et al. Leukemia-associated antigen-specific T-cell responses following combined PRI and WT1 peptide vaccination in patients with myeloid malignancies. *Blood* (2008) 111:236–42. doi:10.1182/blood-2007-08-108241
12. Yasukawa M, Fujiiwa H, Ochi T, Tsumori K, Narumi H, Azuma T, et al. Clinical efficacy of WT1 peptide vaccination in patients with acute myelogenous leukemia and myelodysplastic syndrome. *Am J Hematol* (2009) 84:314–5. doi:10.1002/ajh.21387
13. Maslak PG, Tao T, Krug LM, Channel S, Korotnov T, Zakharova V, et al. Vaccination with synthetic analog peptides derived from WT1 oncogene induces T-cell responses in patients with complete remission from acute myeloid leukemia. *Blood* (2010) 116:171–9. doi:10.1182/blood-2009-10-250993
14. Hashi Y, Sato-Miyashita E, Matsumura R, Kusuda S, Yoshida H, Ohta H, et al. WT1 peptide vaccination following allogeneic stem cell transplantation in pediatric leukemic patients with high risk for relapse: successful maintenance of durable remission. *Leukemia* (2012) 26:530–2. doi:10.1038/leu.2011.226
15. Schmitt M, Schmitt A, Bojowski MT, Chen J, Giannopoulos K, Fei F, et al. Randomized clinical trials are needed to quantify the contribution of WT1 peptide vaccines to clinical responses and disease-free survival. *J Natl Cancer Inst* (2010) 101:1387–90. doi:10.1093/jnci/djp269
16. Gessler M, Pouska A, Cavenese V, Neve RL, Oskin SH, Bruns GA. Homozygous deletion in Wilms tumours of a zinc-finger gene identified by chromosome jumping. *Nature* (1990) 343:774–8. doi:10.1038/343774a0
17. Sugiyama H. Wilms’ tumor gene WT1: its oncogenic function and clinical application. *Int J Hematol* (2001) 73:177–87. doi:10.1023/B:IFOJ.0000002884.19139.e6
18. Davies R, Moore A, Schedl A, Bratt E, Miyahawa K, Ladomery M, et al. The role of WT1 tumor suppressor in the development of epicardium, adrenal gland and throughout nephrogenesis. *Development* (1999) 126:1845–57.
19. Menzsen HD, Renkl HJ, Rodeck U, Maurer J, Notter M, Schwartz S, et al. Presence of Wilms’ tumor gene (wt1) transcripts and the WT1 nuclear protein in the majority of human acute leukemias. *Leukemia* (1995) 9:1060–7.
20. Inoue K, Sugiyama H, Ogawa H, Nakagawa M, Yamagami T, Miwa H, et al. WT1 as a new prognostic factor and a new marker for the detection of minimal residual disease in acute leukemia. *BLOOD* (1994) 84:3071–9.
21. Tamaki H, Ogawa H, Ohyashiki K, Ohyashiki JH, Iwama H, Inoue K, et al. The Wilms’ tumor gene WT1 is a good marker for diagnosis of disease progression. *Leukemia* (1999) 13:393–9. doi:10.1038/sj.leu.2201431
22. Rauscher FJ III. The WT1 Wilms tumor gene product: a developmentally regulated transcription factor in the kidney that functions as a tumor suppressor. *FASEB J* (1993) 7:896–903.
23. Haber DA, Park S, Maheswaran S, Engleman E, Luecke G, Hazen-Martin DJ, et al. WT1-mediated growth suppression of Wilms tumor cells expressing a WT1 splicing variant. *Science* (1993) 262:2057–9. doi:10.1126/science.2620165
24. Sugiyama H. Cancer immunotherapy targeting Wilms’ tumor gene WT1 product, Expert Rev Vaccines (2005) 4:503–12. doi:10.1586/14766558.4.4.503
25. Algar EM, Khorumbly T, Smith SM, Blackburn DM, Bryson GJ, Smith PJ. A WT1 antisense oligonucleotide inhibits proliferation and induces apoptosis in myeloid leukemia cell lines. *Oncogene* (1996) 12:1005–14.
26. Nishida S, Hosen N, Shiraishi T, Kato M, Yanagihara M, Nakatsuka S, et al. AML1-ETO rapidly induces acute myeloblastic leukemia in cooperation with WT1-mediated growth suppression of Wilms tumor cells expressing a WT1 splicing variant. *Science* (1993) 262:2057–9. doi:10.1126/science.2620165
32. Ochsenreither S, Fusi A, Busse A, Bauer S, Scheibenbogen C, Stather D, et al. "Wilms Tumor Protein 1" (WT1) peptide vaccination-induced complete remission in a patient with acute myeloid leukemia is accompanied by the disappearance of a T-cell clone both in blood and bone marrow. *J Immunother* (2011) 34:85–91. doi:10.1097/CJI.0b013e3181f3cc5c

33. Ochsenreither S, Fusi A, Geikowski A, Stather D, Busse A, Stroux A, et al. Wilms’ tumor protein 1 (WT1) peptide vaccination in AML patients: predominant TCR CD3/beta sequence associated with remission in one patient is detectable in other vaccinated patients. *Cancer Immunol Immunother* (2012) 61:313–22. doi:10.1007/s00262-011-1099-y

34. Busse A, Letch A, Scheibenbogen C, Nonnenmacher A, Ochsenreither S, Thiel E, et al. Mutation or loss of Wilms’ tumor gene 1 (WT1) are not major reasons for immune escape in patients with AML receiving WT1 peptide vaccination. *J Transl Med* (2010) 8:3. doi:10.1186/1479-5876-8-3

35. Rezvani K, Brenchley JM, Price DA, Kikuchi Y, Gostick E, Sewell AK, et al. T-cell responses directed against multiple HLA-A*0201-restricted epitopes derived from Wilms’ tumor 1 protein in patients with leukemia and healthy donors: identification, quantification, and characterization. *Clin Cancer Res* (2005) 11:8799–807. doi:10.1158/1078-0432.CCR-05-1314

36. Rezvani K, Brenchley JM, Price DA, Kikuchi Y, Gostick E, Sewell AK, et al. Transfer of PR1-specific T-cell clones from donor to recipient by stem cell transplantation and association with GV activity. *Cytopenogy* (2007) 9:245–51. doi:10.1080/14653240701218524

37. Mollård JF, Lee PP, Kant S, Wieder E, Jiang W, Lu S, et al. Chronic myelogenous leukemia shapes host immunity by selective deletion of high-avidity leukemia-specific T cells. *J Clin Invest* (2003) 111:6309–47. doi:10.1172/JCI250316398

38. Brenchley JM, Karandikar NJ, Betts MR, Ambrozak DR, Hill BF, Crotty LE, et al. Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. *Blood* (2003) 101:2711–20. doi:10.1182/blood-2002-07-2103

39. Zawalesing S, Ferreira Mota SC, Nouta J, Johnson M, Lipford GB, Offringa R, et al. Identification, quantification, and characterization of Wilms’ tumor 1 protein in patients with leukemia and healthy donors: a review of the results of WT1 peptide vaccination strategies for myelodysplastic syndrome and acute myeloid leukemia. *Bone Marrow Transplant* (2015) 49:313–22. doi:10.1038/bmmt.1705057

40. Levy R, Ganjoo KN, Leonard JP, Vose JM, Flinn IW, Ambinder RF, et al. Active idiotypic vaccination versus control immunotherapy for follicular lymphoma. *J Clin Oncol* (2014) 32:797–803. doi:10.1200/JCO.2012.43.9273

41. Falkenburg WJ, Melenhorst JJ, van de Meent M, Kester MG, Hombrink P, et al. Allogeneic HLA-A*02-restricted WT1-specific T cells from mismatched donors are highly reactive but show off-target promiscuity. *J Immunol* (2011) 187:2824–33. doi:10.4049/jimmunol.1100852

42. Marji W, Heemsker M, Kloosterboer FM, Goumy E, Melenhorst JJ, et al. Tumor antigen immunization of sibling stem cell transplant donors in multiple myeloma. *Bone Marrow Transplant* (2005) 36:315–23. doi:10.1038/sj.bmt.1705057

43. Ali OA, Doherty E, Bell WJ, Fradet T, Hudak J, Laliberte MT, et al. Biomaterial-based vaccine induces regression of established intracranial glioma in rats. *Pharm Res* (2011) 28:1074–80. doi:10.1007/s11095-010-0361-x

44. Kaneko S, Mastaglio S, Bondanza A, Ponzoni M, Samvito F, Aldighetti L, et al. IL-7 and IL-15 allow the generation of suicide gene-modified alloreactive self-renewing central memory human T lymphocytes. *Blood* (2009) 113:1086–15. doi:10.1182/blood-2008-05-156059

45. Rapoport AP, Stadtmueller EA, Aqui N, Badros A, Cotte J, Chrisley L, et al. Restoration of immunity in lymphopenic individuals with cancer by vaccination and adoptive T-cell transfer. *Nat Med* (2005) 11:1230–7. doi:10.1038/nm1310

46. Ansell SM, Witzig TE, Kurth MJ, Sloan JA, Jelinek DF, Howell KG, et al. Phase 1 study of interleukin-12 in combination with rituximab in patients with B-cell non-Hodgkin lymphoma. *Blood* (2002) 99:67–74. doi:10.1182/blood.V99.1.67

47. Kohrt HE, Muller A, Baker J, Goldstein MJ, Newell E, Dutt S, et al. Donor immunization with WT1 peptide augments antileukemic activity after MHC-matched bone marrow transplantation. *Blood* (2011) 118:5319–29. doi:10.1182/blood-2011-05-356238

48. Nielapu SS, Munshi NC, Jagannath S, Watson TM, Pennington R, Reynolds C, et al. Tumor antigen immunization of sibling stem cell transplant donors in multiple myeloma. *Bone Marrow Transplant* (2005) 36:315–23. doi:10.1038/sj.bmt.1705057

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 10 October 2014; accepted: 20 January 2015; published online: 04 February 2015.

Citation: Di Stasi A, Jimenez AM, Minagawa K, Al-Obaidi M and Rezvani K (2015) Review of the results of WT1 peptide vaccination strategies for myelodysplastic syndromes and acute myeloid leukemia from nine different studies. *Front. Immunol.* 6:36. doi: 10.3389/fimmu.2015.00036

This article was submitted to Immunotherapeutics and Vaccines, a section of the journal Frontiers in Immunology.

Copyright © 2015 Di Stasi, Jimenez, Minagawa, Al-Obaidi and Rezvani. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.