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PML-RARA–targeted DNA vaccine induces protective immunity in a mouse model of leukemia

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Despite improved molecular characterization of malignancies and development of targeted therapies, acute leukemia is not curable and few patients survive more than 10 years after diagnosis. Recently, combinations of different therapeutic strategies (based on mechanisms of apoptosis, differentiation and cytotoxicity) have significantly increased survival. To further improve outcome, we studied the potential efficacy of boosting the patient's immune response using specific immunotherapy. In an animal model of acute promyelocytic leukemia, we developed a DNA-based vaccine by fusing the human promyelocytic leukemia–retinoic acid receptor-α (*PML-RARA*) oncogene to tetanus fragment C (*FrC*) sequences. We show for the first time that a DNA vaccine specifically targeted to an oncoprotein can have a pronounced effect on survival, both alone and when combined with all-*trans* retinoic acid (ATRA). The survival advantage is concomitant with time-dependent antibody production and an increase in interferon-γ (IFN-γ). We also show that ATRA therapy on its own triggers an immune response in this model. When DNA vaccination and conventional ATRA therapy are combined, they induce protective immune responses against leukemia progression in mice and may provide a new approach to improve clinical outcome in human leukemia.

The effectiveness of a vaccine strategy rests on the acquisition of an immune response that can be both humoral and cell mediated. DNA vaccines have been shown to meet these requirements, leading to strong and persistent cell-mediated and humoral immune responses to the antigen encoded by the plasmid[1]. The application of this type of vaccine in cancer therapy has been successfully used in non-Hodgkin lymphoma patients, using the idiotype of the surface immunoglobulin as the antigen[2,3], and is equally effective in mouse models of lymphoma and myeloma in which protective immunity is observed[4,5]. In myeloid leukemia, fusion proteins resulting from reciprocal translocations may provide a source of specific tumor-associated antigens. PML-RAR-α represents >95% of the fusion proteins in acute promyelocytic leukemia (APL) patients and offers a potential target for DNA vaccination.

We took advantage of the 'APL-transplantable' mouse model, in which cells from the spleen of an APL transgenic mouse (bearing the human *PML-RARA* oncogene) are transplanted into syngeneic recipients[6]. This model mimics human APL, both in its biological characteristics and its response to conventional therapeutic drugs such as ATRA or AS203 (refs. 6–8).

In a preliminary experiment (protocol 1, described in Methods), FVB/N mice ($n = 7$), which were injected with irradiated APL cells for three cycles at 20-d intervals, survived longer than uninjected mice after challenge with APL cells ($P = 0.01$; **Fig. 1a**). One of the mice injected with irradiated APL cells developed antibodies to human PML-RAR-α (**Fig. 2a**) and survived longer than the last surviving antibody-negative mouse of the mice treated with irradiated APL cells, suggesting that proteins from irradiated APL cells could induce an immune response and protect against APL challenge. We then focused our attention on the *PML-RARA* oncoprotein as a potential tumor-associated antigen, and whether a *PML-RARA* DNA plasmid would also protect from leukemia challenge (protocol 2, described in Methods; data not shown). FVB/N mice ($n = 13$) were injected with a *PML-RARA* plasmid for three cycles at 20-d intervals and then challenged with APL cells. We tested full-length *PML-RARA* ($n = 5$) and *PML-RARA-FrC*, a sequence of 105 base pairs around the *PML-RARA* fusion cloned in frame with the highly immunogenic tetanus toxin fragment C ($n = 5$), and compared them with the empty vector ($n = 3$). The longest survival (75 d) was obtained with *PML-RARA-FrC*; the longest survival with the empty vector or the full-length *PML-RARA* was 31 and 33 d, respectively. Thus, vaccination with *PML-RARA-FrC* DNA could, like irradiated APL cells, protect against APL leukemia challenge.

We then designed a protocol that more closely resembled the human APL clinical setting, in which DNA vaccination was initiated after leukemia had been fully established in the mice[6–8] (**Fig. 1b** and **Supplementary Table 1** online). In one trial, 47 leukemic mice were separated into four therapeutic groups: placebo pellets and *PML-*

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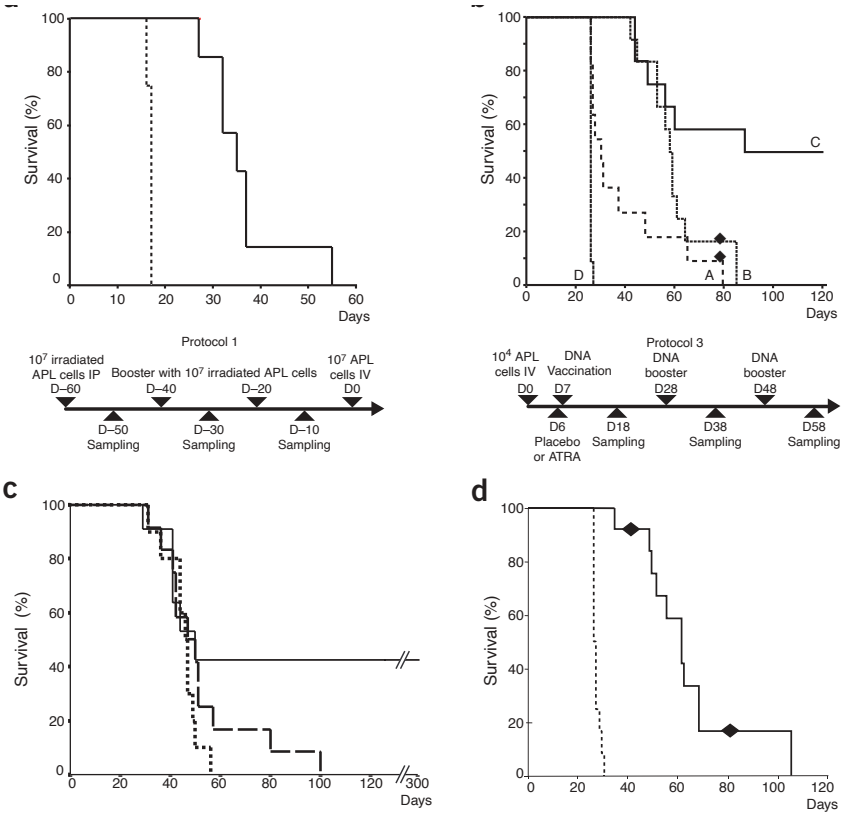

Figure 1 *PML-RARA* DNA vaccination protects against disease progression. (**a**) Kaplan-Meier survival curves and schematic of protocol 1. Mice were challenged with 10^7 APL cells and then treated with irradiated APL cells (solid line; $n = 7$) or not treated (dashed line; $n = 4$). IP, intraperitoneal; IV, intravenous; D, day. (**b**) APL mice were treated with placebo pellets + *PML-RARA-FrC* (A; $n = 11$), ATRA alone (B; $n = 12$), ATRA + *PML-RARA-FrC* (C; $n = 12$) or placebo pellets alone (D; $n = 12$). Schematic of protocol 3 is shown. (**c**) APL mice were treated with ATRA + *PML-RARA-FrC* (solid line; $n = 11$), ATRA alone (dashed line; $n = 12$) or ATRA + empty vector (dotted line; $n = 10$), using protocol 3. (**d**) Mice were treated with placebo (dotted line; $n = 12$) or ATRA (solid line; $n = 12$), together with full-length *PML-RARA*, using protocol 3. Procedural deaths were censored (◆).

RARA-FrC DNA, ATRA pellets only, ATRA pellets and *PML-RARA-FrC* DNA, and placebo pellets only (**Fig. 1b**). Six of 11 (56%) mice of the placebo and *PML-RARA-FrC* group had significantly extended survival compared with the placebo-only group ($P < 0.001$ by Wilcoxon test), indicating an antileukemic effect of *PML-RARA-FrC*. When ATRA and *PML-RARA-FrC* were combined, survival was superior to that obtained with ATRA alone ($P = 0.013$ by log rank test). Six of 12 mice in the ATRA plus DNA group survived up to 120 d, whereas only one mouse of the ATRA-only group lived up to 85 d. In a second trial, we showed that antileukemic efficacy required the presence of *PML-RARA*. We assigned 33 leukemic mice to three groups: ATRA alone, ATRA and *PML-RARA-FrC*, and ATRA and empty vector. Extended survival was only obtained when the *PML-RARA* sequence was present (**Fig. 1c**). Mice treated with ATRA anf FrC had the same survival as mice treated with ATRA alone (data not shown).

Taken together, these results show that treatment with both ATRA and *PML-RARA-FrC* provides a significant survival advantage in leukemic mice. The significance was even more apparent when the two cohorts treated according to protocol 3 (**Fig. 1b,c**) were pooled. After 120 d, 44% of the mice in the ATRA and *PML-RARA-FrC* group were still alive, compared with none in the group treated with ATRA and *PML-RARA* without *FrC* ($P < 0.01$; **Supplementary Table 1** online). These studies underline the requirement for both *PML-RARA* and *FrC* and the survival advantage provided by combining *PML-RARA-FrC* DNA vaccination with ATRA therapy in this APL model.

To assess the mechanisms responsible for the prolonged survival induced by the DNA vaccine, we monitored mice for antibody production and cell-mediated immune responses. Sera from mice treated with irradiated APL cells (**Fig. 1a**), ATRA alone, ATRA and *PML-RARA-FrC* or full-length *PML-RARA* (**Fig. 1b,d**) reacted with human PML-RAR-α (**Fig. 2a–c**), human RAR-α (**Fig. 2a,c**), recombinant human glutathione *S*-transferase (GST)-RAR-α (**Fig. 2d**) and mouse endogenous RAR-α (**Fig. 2b**). Sera from FVB/N or placebo-treated mice did not detect PML-RAR-α or RAR-α (**Fig. 2a** and data not shown).

Because some of the antibodies that were produced recognized a moiety of RAR-α present in both RAR-α and PML-RAR-α, we set up an ELISA to measure RAR-α antibody production. Sera from mice treated according to protocol 3 (**Fig. 1b**; $n = 47$) were analyzed. Both the number of mice with a positive ELISA result and the amount of antibody produced by the mice increased with duration of treatment (**Table 1**). Although no antibody was found in mice treated with placebo or *PML-RARA-FrC* DNA alone, perhaps because death might have occurred before detectable antibody production (**Table 1**), substantial antibody production was detected in mice treated with ATRA alone, suggesting for the first time that ATRA therapy can enhance an antibody response (**Fig. 2b** and **Table 1**). Preincubating the sera with 5- or 20-fold higher concentrations of recombinant GST-RAR-α protein reduced detection of RAR-α antibody from 4% to 30%, respectively (data not shown). In a retrospective analysis, mice in which an antibody to RAR-α could be detected by ELISA showed a significant survival advantage (**Fig. 2e**; $P < 0.001$).

Table 1 Antibody to RAR-α increases with duration of treatment

		Day 18	Day 38	Day 58
Placebo	Ab-positive[a]	0/12	No survivors	No survivors
Placebo + PML-RAR-α-FrC	Ab-positive[a]	0/11	0/2	0/1
ATRA	Ab-positive[a]	0/12	7/12 (58%)	4/5 (80%)
	Mean ± s.d.[b]	NA	2.22 ± 1.18	13.03 ± 5.41
	Median[b]	NA	1.95	15.09
ATRA + PML-RAR-α-FrC	Ab-positive[a]	1/12 (8%)	5/12 (42%)	7/7 (100%)
	Mean ± s.d.[b]	1.79	2.35 ± 1.71	3.78 ± 3.9
	Median[b]	NA	1.82	2.44

Mice are same as those in **Figure 1b**. NA, not applicable. [a]Number of mice with detectable antibodies per number of mice alive at that date. [b]Values are given as mean ± s.d. and median of U_A from antibody (Ab)-positive mice.

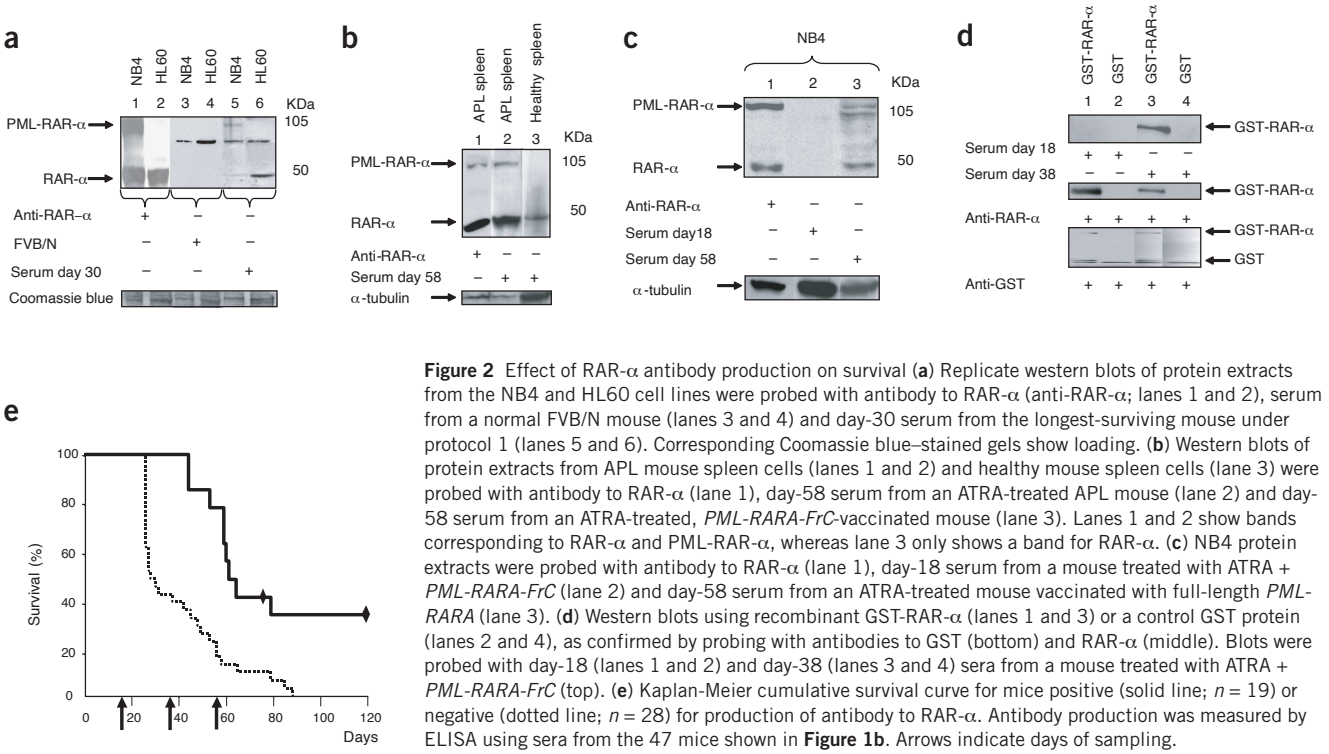

Figure 2 Effect of RAR-α antibody production on survival (**a**) Replicate western blots of protein extracts from the NB4 and HL60 cell lines were probed with antibody to RAR-α (anti-RAR-α; lanes 1 and 2), serum from a normal FVB/N mouse (lanes 3 and 4) and day-30 serum from the longest-surviving mouse under protocol 1 (lanes 5 and 6). Corresponding Coomassie blue–stained gels show loading. (**b**) Western blots of protein extracts from APL mouse spleen cells (lanes 1 and 2) and healthy mouse spleen cells (lane 3) were probed with antibody to RAR-α (lane 1), day-58 serum from an ATRA-treated APL mouse (lane 2) and day-58 serum from an ATRA-treated, *PML-RARA-FrC*-vaccinated mouse (lane 3). Lanes 1 and 2 show bands corresponding to RAR-α and PML-RAR-α, whereas lane 3 only shows a band for RAR-α. (**c**) NB4 protein extracts were probed with antibody to RAR-α (lane 1), day-18 serum from a mouse treated with ATRA + *PML-RARA-FrC* (lane 2) and day-58 serum from an ATRA-treated mouse vaccinated with full-length *PML-RARA* (lane 3). (**d**) Western blots using recombinant GST-RAR-α (lanes 1 and 3) or a control GST protein (lanes 2 and 4), as confirmed by probing with antibodies to GST (bottom) and RAR-α (middle). Blots were probed with day-18 (lanes 1 and 2) and day-38 (lanes 3 and 4) sera from a mouse treated with ATRA + *PML-RARA-FrC* (top). (**e**) Kaplan-Meier cumulative survival curve for mice positive (solid line; $n = 19$) or negative (dotted line; $n = 28$) for production of antibody to RAR-α. Antibody production was measured by ELISA using sera from the 47 mice shown in **Figure 1b**. Arrows indicate days of sampling.

To evaluate the involvement of T cells in the observed protection, $CD4^+$ and $CD8^+$ cells were monitored in mice treated with ATRA alone or with *PML-RARA-FrC* (**Fig. 1c**) and the results were analyzed according to the survival response achieved (see Methods). On day 18 after the first DNA injection, mice with complete remission or partial responses had relatively higher numbers of $CD4^+$ and $CD8^+$ T cells compared with nonresponders (**Fig. 3a**). Mice with complete remission maintained normal counts up to 112 d after the first injection of PML-RAR-α-FrC (data not shown). The increase in these cell populations preceded the detection of RAR-α antibody. Although these data are not significant, they show an increase in T cells that may help predict survival.

We next conducted proliferation and cytokine release assays on long-term survivors (**Fig. 1c**). Although spleen cells from mice treated with ATRA and *PML-RARA-FrC* proliferated in the presence of allogeneic BALB/c spleen cells, they did not proliferate more than control cells when stimulated with syngeneic APL cells (data not shown). In contrast, a specific sixfold increase in IFN-γ after 48 h of culture was observed when spleen cells treated with ATRA and *PML-RARA-FrC* were stimulated with irradiated APL cells, compared with spleen cells from healthy mice treated with ATRA only (327 pg/ml versus 52 pg/ml; $P < 0.02$; **Fig. 3b**). No significant differences were observed for other cytokines such as interleukin-2, interleukin-4, interleukin-5 and tumor necrosis factor-α. Although the natural killer cell population was increased in APL mice with or without treatment, no difference in natural killer cell activity was detected (data not shown). Likewise, we were unable to detect significant cytotoxic T-cell responses to mouse APL spleen cells. These cellular functions could be defective in this model, or their absence might be related to the low frequency of these effectors (CTLs from vaccinated mice), a phenomenon often reported in mice and humans in the DNA vaccine setting.

This study suggests that enhancement of an immune response by PML-RAR-α-FrC is responsible for the prolonged survival of treated mice. Because irradiated APL cells alone can elicit an antibody response, and because we have shown a specific antibody response against PML-RAR-α, it is likely that this immune effect was triggered by the APL cells. Other animal trials with various DNA or peptide vaccines have shown production of specific antibodies[4,5] implicating $CD4^+$ T cells, especially in trials using the FrC construct[4,9,10]. $CD4^+$ T cells are implicated in establishing immune memory[11] and may explain the late and prolonged effect of DNA vaccination and ATRA treatment on survival and IFN-γ secretion. Thus, antibody production, T-cell response and cytokine release strongly suggest that a

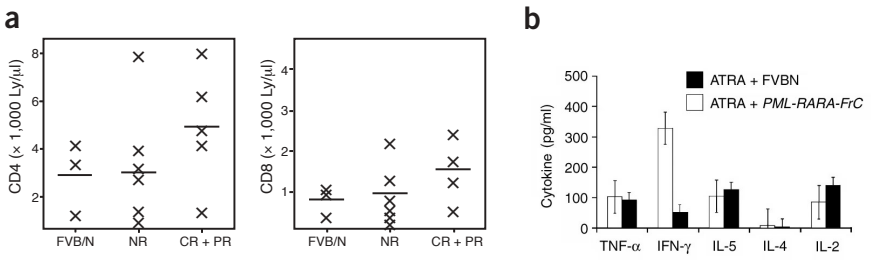

Figure 3 Effect of *PML-RARA* DNA vaccination on $CD4^+$ and $CD8^+$ T-cell numbers, cytokine secretion and survival. (**a**) $CD4^+$ and $CD8^+$ levels and response to treatment of mice shown in Figure 1c, on day 18. Absolute numbers are shown. Horizontal bars show the means of the nonresponders (NR), partial responders (PR) and complete responders (CR). Ly, lymphocytes; FVB/N, untreated healthy mice. (**b**) Histogram showing increase in IFN-γ in APL mice after treatment with ATRA + *PML-RARA-FrC*, as compared with healthy FVB/N mice treated with ATRA. Data are shown as mean ± s.e.m. ($n = 3$) after stimulation with irradiated APL cells.

CD4+ T-lymphocyte response predominates in our DNA vaccination model. Nevertheless, IFN-γ also mediates CD8+ cell function and could explain the sustained CD8+ levels seen in the responding mice. Although specific cytotoxic T cells could not be detected, the role of CD8+ cytotoxic T cells in the survival advantage cannot be ruled out. Adaptive immunity has been shown to cooperate with ATRA to induce and maintain remission in mouse APL[12].

Little is known about the immune response of human APL patients towards APL cells, although the limited data that are available are in accordance with our results[13,14]. It is not yet known whether the endogenous or exogenous PML-RAR-α protein can be correctly processed and presented by either the APL cell itself or by antigen-presenting cells, through major histocompatibility class I molecules.

Breaking the tolerance to PML-RAR-α using a vaccine approach could induce autoimmunity, as most tumor-associated oncoproteins can generate self antigens[15,16]. The results of various immunization trials in mice did not include any major autoimmunity lesions[17]. Mice treated with DNA and ATRA that produced antibody to RAR-α were as healthy and survived (120–300 d) about as long as RARA$^{-/-}$, which survive to adulthood[18,19]. In human trials, autoimmunity has not yet been limiting in initiating treatment, but needs to be cautiously monitored in new trials.

Conventional ATRA-based APL therapy regimens already efficiently induce complete remission in 95% of human APL, but DNA vaccination may provide added value in the control of minimal residual disease. This is also the time when vaccination may prove most effective, as we have observed that the immune response and efficacy of the DNA vaccination correlated with tumor burden. Our results, however, strongly stress that DNA vaccination should be combined with ATRA. The adjuvanticity of vitamin A is already well documented[20]. In addition to PML-RAR-α and RAR-α degradation[21], ATRA induces antigen shedding in APL cells, which probably boosts immune responses[22]. Another mechanism of relevance, raised by this study and others, involves the use of an immunogenic foreign protein to boost antitumor response. Transgenic mouse models have been extensively used to study the efficacy of DNA-based vaccines. Most of them have been in a heterologous setting, such as ours, where the transgene is a human oncogene or gene coding for a self tumor-associated antigen, and the immunogen is a human DNA sequence of the same transgene[4,5]. Some DNA vaccines have been successfully translated into clinical trials in an autologous setting (unpublished data). Our present model may differ from others in that the *RARA* sequence of the DNA vaccine is 92% homologous at the nucleotide level and 100% homologous at the amino acid level to the mouse *Rara* sequence, and may well be immunogenic, as suggested by the induction of antibodies against mouse RAR-α. These observations suggest that this model may be applied in an autologous setting, and imply that a human DNA vaccine may work in human APL. Nevertheless, it may be beneficial to take advantage of a foreign protein or DNA sequence to boost responses in humans[23,24]. These strategies will be explored in the future, but as proof of principle, this model was chosen to investigate the feasibility of inducing immunity against a tumor-specific human fusion protein using DNA delivery. Evidence of induction has been obtained, and the ability of the induced antibodies to bind autologous protein has been demonstrated, indicating that tolerance has been broken to some extent. The present study shows for the first time that ATRA, in combination with a vaccine of specific tumor-associated antigen DNA fused to tetanus fragment C, may prolong clinical remission by boosting immune responses against tumor antigens.

METHODS

Animal model. All procedures complied with European or national regulations. Transgenic mice bearing the human *PML-RARA* cDNA were previously constructed in the FVB/N inbred strain of mice. A transplant model was established in which 100% of the mice die of the disease, compared with 30% of the transgenic animals[6]. This fusion presents a high antigenic index[14]. Establishment of leukemia was assessed by the appearance of high leukocyte and low platelet counts at around day 8 (ref. 6; Hemavet blood counter, CDC Technologies). Enlargement of organs and tissue section examination confirmed that the cause of death was APL[7]. RT-PCR was conducted to confirm the presence of *PML-RARA* transcripts in spleen cells and to assess tumor load[25,26]. ATRA and placebo (5-mg, 21-d-release pellets; Innovative Research) were administered subcutaneously[6]. We challenged mice with 10^4, 10^6 and 10^7 APL cells.

Vaccination protocols. We directionally cloned 105 base pairs of the sequence around the *PML-RARA* fusion, together with a peptide signal and the *FrC* sequence, into a $pCNA_3$-based vector (clone YJFrC; ref. 4) using a Seamless cloning kit (Stratagene), to produce *PML-RARA-FrC*. We used the following primer sequences: *PML-RARA* sense, 5′-ACTGCTCTTCCTCCGAGGTCTTCCTGCCCAACAGC-3′; *PML-RARA* antisense, 5′-ACTGCTCTTCCTTTCGAGGGAGGGCTGGGCACTAT-3′; YJFC leader antisense, 5′-ACTGCTCTTCCGGAGTGGGCCCCCGGGGCCAC-3′; *FrC* sense, 5′-ACTGCTCTTCCAAAAACCTTGATTGTTGGGTC-3′. The construct was verified by sequencing (at the University of California, San Francisco, facility) and expression was confirmed by transient transfection into COS cells (data not shown). Plasmid DNA preparations were then purified for vaccination[26]. Full-length *PML-RARA* was also used[27].

For vaccination protocol 1, APL cells were irradiated (25 Gy) for use as 'cellular therapy' to protect against leukemia challenge. We injected 10^7 cells intravenously into syngeneic FVB/N mice ($n = 7$) at 20-d intervals for three cycles, starting on day –60. Sixty days after the first injection (termed 'day 0 of therapy'), mice were challenged with 10^7 APL cells, injected intravenously, and monitored for survival.

For vaccination protocol 2, *PML-RARA* plasmids were used as DNA vaccines to protect against leukemia challenge. DNA was injected into syngeneic FVB/N mice ($n = 13$) at 20-d intervals for three cycles, starting on day –60. Sixty days after the first injection (termed 'day 0 of therapy'), mice were challenged with 10^4 APL cells, injected intravenously, and monitored for survival.

For vaccination protocol 3, *PML-RARA* plasmids alone or in combination with ATRA were tested as DNA vaccines in the setting of established APL leukemia. APL cells were injected intravenously on day 0. A week later, placebo or ATRA pellets were introduced subcutaneously. The following day, DNA was injected at 20-day intervals for three cycles.

For each protocol, we injected 50 μg of DNA in HBSS intramuscularly into the quadriceps of each mouse. Blood samples were collected by retro-orbital bleeding 10 d after each course of injection. Survival was analyzed by the Kaplan-Meier method; comparisons between groups were analyzed by the Wilcoxon and log rank tests. Comparisons between groups for antibody response and cytokine release were analyzed using the Student *t*-test.

Measurement of immune response. Antibodies to RAR-α were detected by ELISA using purified GST-RAR-α. Each serum was tested in duplicate. Specific absorbance (A_{spe}) was calculated as the difference between the mean absorbance with or without GST-RAR-α. Mouse monoclonal antibody to RAR-α (9αF), diluted 1:200,000, served as a positive control (medianA_{spe} = 0.094). To normalize the results, the specific absorbance obtained for each sample was divided by the specific absorbance of the positive control and expressed as arbitrary units, U_A ((U_A of sample) = (A_{spe} of sample) ÷ (A_{spe} of positive control)). A serum was considered positive if U_A was greater than 1. Inter- and intra-assay variations were <30% and 10%, respectively.

Western blot analysis was done using protein extracts from the NB4 human APL cell line (PML-RAR-α-positive), the HL60 human acute myelogenous leukemia cell line (PML-RAR-α-negative), FVB/N or mouse APL spleen cells, GST from pGEX and human GST-RAR-α from pGEXRARα[28,29]. Membranes were probed with mouse serum (1:50) or antibody 9αF to RAR-α (1:500–1:5,000) and reprobed with monoclonal antibodies to α-tubulin

(1:1,000; Sigma), RAR-α (9αF) or GST (1:1,000; Santa Cruz Biotechnology). Immunoreactive proteins were revealed using an enhanced chemiluminescence detection kit (Amersham). Exposure times were 1 min for antibody to RAR-α and 3 min for FVB/N and day-30 sera.

Cell-mediated immune response. We assayed 5×10^5 spleen cells for [^{3}H]thymidine incorporation, and collected the supernatants for cytokine release assays after incubation with 1×10^6 irradiated spleen cells from FVB/N, APL or BALB/c mice. Cytokine release was measured using a T_H1/T_H2 cytokine bead array kit (PharMingen). CD4, CD8, CD45 and CD3 were evaluated with specific FITC- or phycoerythrin-conjugated monoclonal antibodies (PharMingen). Cells were analyzed using a FACSCalibur cytometer and CellQuest software (Becton Dickinson). Mice defined as nonresponders survived up to 60 d, partial responders survived 60–120 d and complete responders survived >120 d. Cytotoxic T cells were generated by coculturing spleen cells from vaccinated, untreated or BALB/c mice with irradiated APL cells and tested in the standard ^{51}Cr-release assay. Natural killer cell activity was evaluated by ^{51}Cr-release assay using YAC cell lines as target cells[30].

Note: Supplementary information is available on the Nature Medicine website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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1. Wolff, J.A. *et al.* Direct gene transfer into mouse muscle *in vivo*. *Science* **247**, 1465–1468 (1990).
2. Stevenson, F.K. *et al.* Idiotypic DNA vaccines against B-cell lymphoma. *Immunol. Rev.* **145**, 211–228 (1995).
3. Syrengelas, A.D., Chen, T.T. & Levy, R. DNA immunization induces protective immunity against B-cell lymphoma. *Nat. Med.* **2**, 1038–1041 (1996).
4. King, C.A. *et al.* DNA vaccines with single-chain Fv fused to fragment C of tetanus toxin induce protective immunity against lymphoma and myeloma. *Nat. Med.* **4**, 1281–1286 (1998).
5. Spellerberg, M.B. *et al.* DNA vaccines against lymphoma: promotion of anti-idiotypic antibody responses induced by single chain Fv genes by fusion to tetanus toxin fragment C. *J. Immunol.* **159**, 1885–1892 (1997).
6. Brown, D. *et al.* A PMLRARα transgene initiates murine acute promyelocytic leukemia. *Proc. Natl. Acad. Sci. USA* **94**, 2551–2556 (1997).
7. Lallemand-Breitenbach, V. *et al.* Retinoic acid and arsenic synergize to eradicate leukemic cells in a mouse model of acute promyelocytic leukemia. *J. Exp. Med.* **189**, 1043-52 (1999).
8. Kogan, S.C., Hong, S.H., Shultz, D.B., Privalsky, M.L. & Bishop, J.M. Leukemia initiated by PMLRARα: the PML domain plays a critical role while retinoic acid-mediated transactivation is dispensable. *Blood* **95**, 1541–1550 (2000).
9. Bogen, B. Peripheral T cell tolerance as a tumor escape mechanism: deletion of CD4$^+$ T cells specific for a monoclonal immunoglobulin idiotype secreted by a plasmacytoma. *Eur. J. Immunol.* **26**, 2671–2679 (1996).
10. Rice, J., Elliott, T., Buchan, S. & Stevenson, F.K. DNA fusion vaccine designed to induce cytotoxic T cell responses against defined peptide motifs: implications for cancer vaccines. *J. Immunol.* **167**, 1558–1565 (2001).
11. Chen, S.A. *et al.* Induction of antitumor immunity with combination of HER2/neu DNA vaccine and interleukin 2 gene-modified tumor vaccine. *Clin. Cancer Res.* **6**, 4381–4388 (2000).
12. Westervelt, P. *et al.* Adaptive immunity cooperates with liposomal all-trans-retinoic acid (ATRA) to facilitate long-term molecular remissions in mice with acute promyelocytic leukemia. *Proc. Natl. Acad. Sci. USA* **99**, 9468–9473 (2002).
13. Dermime, S. *et al.* Lack of T-cell-mediated recognition of the fusion region of the pml/RAR-α hybrid protein by lymphocytes of acute promyelocytic leukemia patients. *Clin. Cancer Res.* **2**, 593–600 (1996).
14. Gambacorti-Passerini, C. *et al.* Human CD4 lymphocytes specifically recognize a peptide representing the fusion region of the hybrid protein pml/RAR α present in acute promyelocytic leukemia cells. *Blood* **81**, 1369–1375 (1993).
15. Finn, O.J. & Forni, G. Prophylactic cancer vaccines. *Curr. Opin. Immunol.* **14**, 172–177 (2002).
16. Forni, G., Lollini, P.L., Musiani, P. & Colombo, M.P. Immunoprevention of cancer: is the time ripe? *Cancer Res.* **60**, 2571–2575 (2000).
17. Hu, J. *et al.* An evaluation of the potential to use tumor-associated antigens as targets for antitumor T cell therapy using transgenic mice expressing a retroviral tumor antigen in normal lymphoid tissues. *J. Exp. Med.* **177**, 1681–1690 (1993).
18. Lufkin, T. *et al.* High postnatal lethality and testis degeneration in retinoic acid receptor α mutant mice. *Proc. Natl. Acad. Sci. USA* **90**, 7225–7229 (1993).
19. Mark, M. *et al.* A genetic dissection of the retinoid signalling pathway in the mouse. *Proc. Nutr. Soc.* **58**, 609–613 (1999).
20. Dresser, D.W. Adjuvanticity of vitamin A. *Nature* **217**, 527–529 (1968).
21. Zhu, J. *et al.* Retinoic acid induces proteasome-dependent degradation of retinoic acid receptor α (RARα) and oncogenic RARα fusion proteins. *Proc. Natl. Acad. Sci. USA* **96**, 14807–14812 (1999).
22. Stephensen, C.B. *et al.* Vitamin A enhances *in vitro* Th2 development via retinoid X receptor pathway. *J. Immunol.* **168**, 4495–4503 (2002).
23. McConkey, S.J. *et al.* Enhanced T-cell immunogenicity of plasmid DNA vaccines boosted by recombinant modified vaccinia virus Ankara in humans. *Nat. Med.* **9**, 729–735 (2003).
24. Krieg, A.M. CpG motifs: the active ingredient in bacterial extracts? *Nat. Med.* **9**, 831–835 (2003).
25. Cassinat, B. *et al.* Quantitation of minimal residual disease in acute promyelocytic leukemia patients with t(15;17) translocation using real-time RT-PCR. *Leukemia* **14**, 324–328 (2000).
26. Sambrook, J., Fritsch, E.F. & Maniatis, T. Plasmid vectors. in *Molecular Cloning. A Laboratory Manual,* 1.33– 1.52 (Cold Spring Harbor Laboratory Press, New York, 1989).
27. de The, H. *et al.* The PML-RAR α fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. *Cell* **66**, 675–684 (1991).
28. Delva, L. *et al.* Physical and functional interactions between cellular retinoic acid binding protein II and the retinoic acid-dependent nuclear complex. *Mol. Cell. Biol.* **19**, 7158–7167 (1999).
29. Parrado, A. *et al.* Retinoic acid receptor α1 variants, RARα1δB and RARα1δBC, define a new class of nuclear receptor isoforms. *Nucleic Acids Res.* **29**, 4901–4908 (2001).
30. Lust, J.A., Kumar, V., Burton, R.C., Bartlett, S.P. & Bennett, M. Heterogeneity of natural killer cells in the mouse. *J. Exp. Med.* **154**, 306–317 (1981).