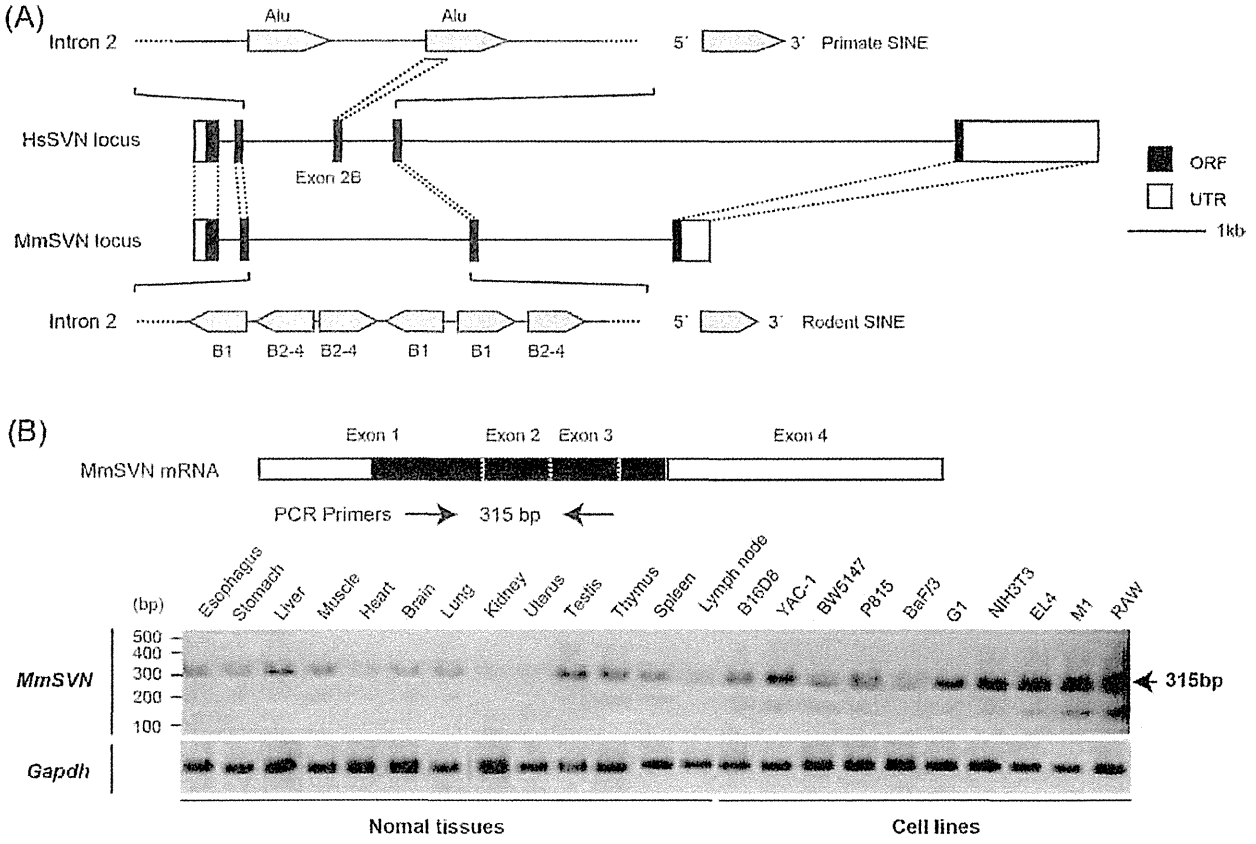

Fig. 1. Genome structure and expression of human and murine SVN gene. (A) Comparison of human and murine survivin gene structure. Survivin gene structures were defined by the Ensembl genome browser. Primate and rodent SINEs were predicted using Repeat Masker program. Filled boxes, coding regions; open boxes, 5′- and 3′-untranslated regions. (B) Structure of murine survivin transcript and RT-PCR analysis of organs and cell lines. Arrows, survivin-detecting PCR primers.

Fig. 2. Structure and purification of chimeric MmSVN2B protein. (A) Strategy for constructing chimeric MmSVN2B protein. Human exon 2B was inserted into MmSVN by PCR. (B) Alignment of murine and human SVN sequences. Black shaded area, residues conserved between human and murine SVN; Hs, human; Mm mouse. *, $MmSVN_{13\text{-}27}$/$HsSVN_{13\text{-}27}$ peptide; +, Hs/Mm $SVN_{53\text{-}67}$ peptide; #, SVN2B peptide. (C) Purification of N-His-tagged MmSVN2B and HsSVN2B proteins. N-His-tagged SVN proteins were purified using a Profinia protein purification system from BL21 (DE3) competent cells. Purified SVN protein buffer was sequentially exchanged to PBS containing 2 M urea.

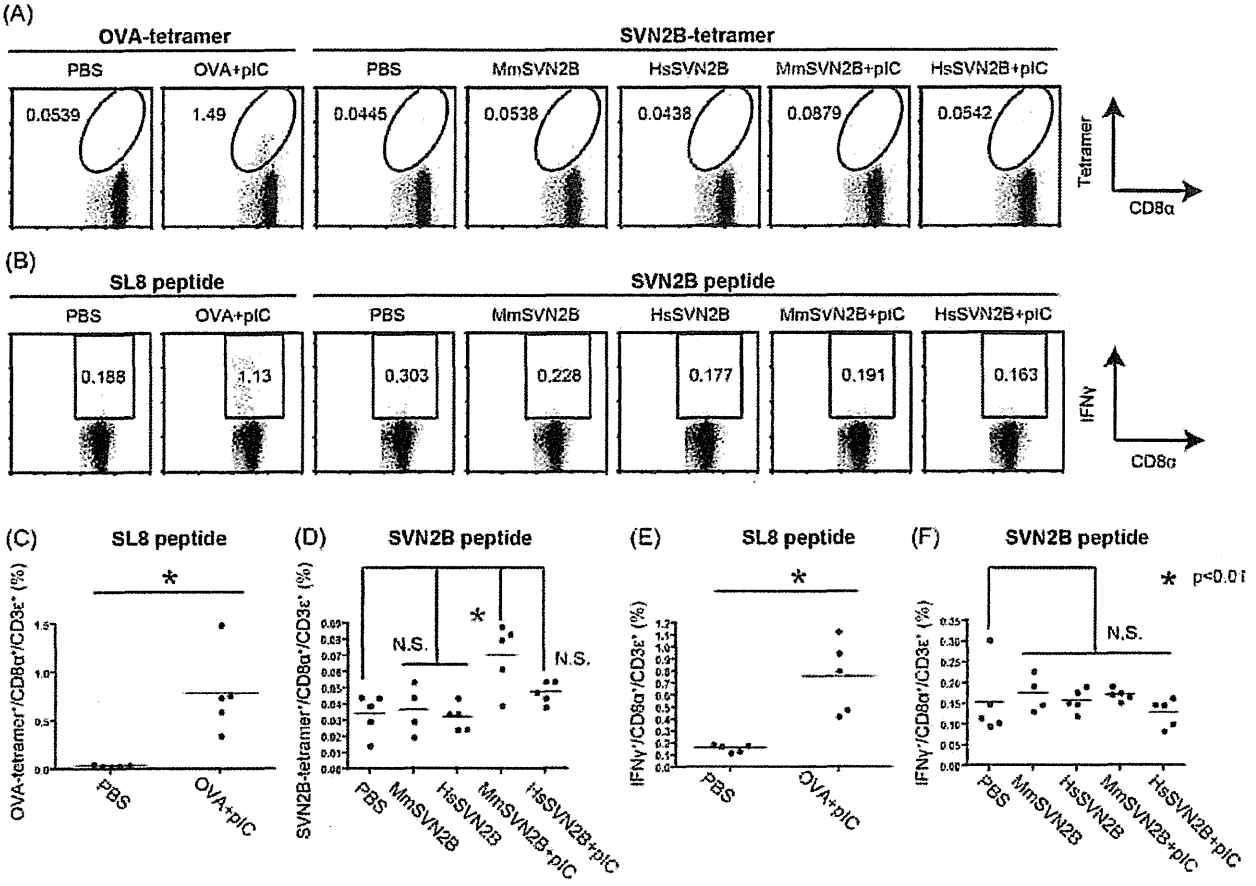

Fig. 3. Expansion of OVA and SVN-specific CD8⁺ T cells. (A) HLA24^b-Tg mice were immunized with 100 μg antigen and 100 μg poly I:C once a week for 4 weeks. After 7 days from the last immunization, spleens were homogenized and stained with FITC-CD8α and PE-OVA or PE-survivin tetramer to detect antigen-specific CD8⁺ T cells. (B) Splenocytes were cultured *in vitro* in the presence of SL8 or SVN2B peptides for 6 h and IFN-γ production was measured by FACS. (C, D) Average percentages of OVA-positive and SVN2B-tetramer positive CD8⁺ T cells shown in (A). (E, F) Average percentages of IFN-γ producing CD8⁺ T cells specifically in response to SL8 or SVN2B peptide in (B). *$p < 0.01$.

(Charalambous et al. 2006). His-tagged MmSVN2B and HsSVN2B proteins were purified and used as Ags (Fig. 2C).

CD4⁺ and CD8⁺ T cells that react to MmSVN2B plus polyI:C

We examined the ability of MmSVN2B to induce IFN-γ and CD8⁺ T cell proliferation by immunizing HLA24^b-Tg mice with MmSVN2B or HsSVN2B with or without polyI:C (Fig. 3). SVN2B-specific CTLs were probed by SVN2B-tetramer (Fig. 3A) and IFN-γ staining (Fig. 3B). SVN2B-specific human CD8⁺ T cells were detected with SVN2B-tetramer (Fig. S1), which enabled us to search for SVN2B-specific CTLs in HLA24^b-Tg mice (Idenoue et al. 2005). Expression of CD40 was up-regulated in CD8α⁺ conventional DCs to a similar extent with MmSVN2B or HsSVN2B (Fig. S2), consistent with a report on CD40 that promotes cross-priming by Ahonen et al. (J Exp Med, 2004). OVA and polyI:C were used as positive controls (Fig. 3A, B left panels), and SL8 (SIINFEKL)-specific CTLs were monitored with OVA tetramer (Azuma et al. 2012). Both OVA-tetramer-positive and IFN-γ-producing CD8⁺ T cells were detected in mice immunized with OVA and polyI:C (Fig. 3C, E). Without polyI:C stimulation, only small number of OVA-tetramer-positive cells were upregulated compared to controls (Azuma et al. 2012; Azuma & Seya unpublished data).

When HLA24^b-Tg mice were immunized with MmSVN2B or HsSVN2B without polyI:C, no significant induction of SVN2B-tetramer-positive (Fig. 3D) or IFN-γ-inducing cells was observed (Fig. 3F). When polyI:C was included, only a small increase in SVN2B-tetramer-positive cells was detected in mice given MmSVN + polyI:C with no significant increase in IFN-γ (Fig. 3F). Mice receiving HsSVN + polyI:C (Fig. 3D) or polyI:C alone (not shown) showed no significant increase in SVN2B-specific CD8⁺ T cells. Consistent with the lack of tetramer-positive CTL induction, MmSVN2B treatment failed to regress MmSVN2B-transfected tumor cells implanted into HLA24^b-Tg mice. In EG7 tumor-bearing mice, administration of polyI:C alone (without Ag) induces tumor-growth retardation due to the contribution of endogenous Ag (Azuma et al. 2012), but in this case with tumor-unloaded mice polyI:C exhibited no tumor-regressing activity (data not shown), possibly due to the lack of Ag.

Next, we determined the amounts of CD4⁺ T cells that reacted with MmSVN2B. The positive control group received OVA Ag and polyI:C (Fig. 4A, B). The negative control group received PBS without Ag and polyI:C, but basal frequencies of IFN-γ-producing CD4⁺ T cells were detected in this group even in the absence of polyI:C or Ag (Fig. 4). When MmSVN2B or HsSVN2B only was used to immunize mice, no significant response was seen in CD4⁺ T cells compared to PBS controls (Fig. 4A, C–E). When polyI:C was included, IFN-γ-producing CD4⁺ T cells restimulated with Hs/MmSVN$_{53-67}$ peptide increased significantly in mice that received MmSVN and HsSVN (Fig. 4C, D). The sequence of MmSVN$_{53-67}$ was identical to the sequence of HsSVN$_{53-67}$ (Fig. 2B). However, we did not detect a significant increase in IFN-γ-producing CD4⁺ T cells in mice

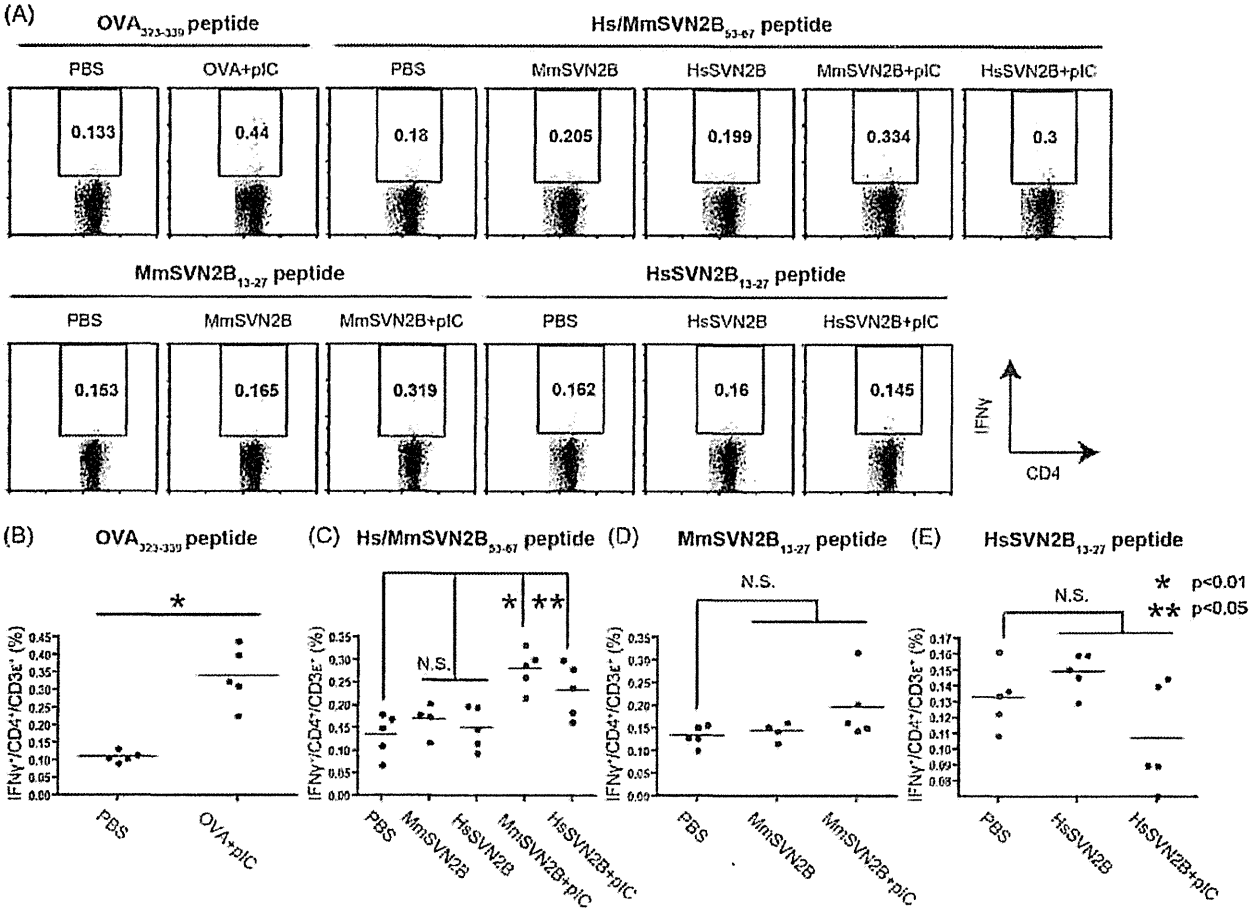

Fig. 4. Expansion of OVA and SVN-specific CD4+ T cells. (A) HLA24b-Tg mice were immunized with 100 μg each antigen and 100 μg poly I:C once a week for 4 weeks. After 7 days from the last immunization, splenocytes were cultured with 100 nM $OVA_{323-339}$ peptide or SVN helper peptide for 6 h, and 10 μg/ml brefeldin A (Sigma–Aldrich) was added in the last 5 h. After cell surface and intracellular staining, IFN-γ production of CD4+ T cells was measured by FACS. Average percentages of IFN-γ-producing CD4+ T cells in response to (B) $OVA_{323-339}$ peptide; (C) Hs/Mm SVN_{53-67} peptide; (D) $MmSVN_{13-27}$ peptide; (E) $HsSVN_{13-27}$ peptide. $^{*}p < 0.01$, $^{**}p < 0.05$.

restimulated with $MmSVN_{13-27}$ or $HsSVN_{13-27}$ peptide (Fig. 4D, E). Differences in these two CD4 epitope sequences are in Fig. 2B.

Ab production by immunization with MmSVN2B with polyI:C

Activation of Th1 cells is essential for B cell antibody class switching. Therefore, we examined production of SVN-specific Ab in Tg mice that did or did not receive polyI:C. Serum was collected from HLA24b-Tg mice immunized with different Ags and polyI:C. OVA and polyI:C were the positive control and resulted in a significant increase in OVA-specific IgG1, IgG2a and IgG2b by ELISA (Fig. 5 left panels). When HLA24b-Tg mice were immunized with MmSVN2B or HsSVN2B without polyI:C, no significant production of any isotypes was observed (Fig. 5 center and right panels). When polyI:C was included, MmSVN2B or HsSVN2B-specific isotypes increased significantly.

Discussion

We demonstrated that HLA24b-Tg mice induced $Hs/MmSVN_{53-67}$-specific CD4+ T cells and SVN-specific Ab followed by Th1 cell activation in response to injection of polyI:C and MmSVN2B protein. This result was partly inconsistent with a previous report (Charalambous et al. 2006) using Balb/c mice and HsSVN conjugated to Dec205 mAb. That is, our study with C57BL/6 mice and MmSVN2B did not detect significant increases in $MmSVN_{13-27}$-specific CD4+ T cells after subcutaneous injection of MmSVN2B with polyI:C. Thus, the xenogeneic differences in sequence between HsSVN and MmSVN did not always contribute to generating effective CD4+ T cells specific for a tumorigenic protein in C57BL/6 mice. The haplotype of the MHC class II proteins between Balb/c (having H-2d) and C57BL/6 mice (having H-2b) and Dec205 mAb conjugation (Charalambous et al. 2006) might be the reason for these different results. However, no CD8+ CTLs against the 2B peptide were detected even when using a specific tetramer for detection of CD8+ CTLs (Fig. S1). Hence, polyI:C was required for proliferation of self-reactive CD4+ Th1 cells that recognized the syngeneic epitope without proliferation of SVN2B peptide-specific CTLs.

OVA were used as positive controls (Fig. 3A, B left panels), and SL8 (SIINFEKL)-specific CTLs were monitored with OVA tetramer (Azuma et al. 2012). Here, T cell activation by polyI:C + MmSVN2B is a focus in this study. However, there is a lot-to-lot difference of T cell-activating activity in polyI:C + OVA as in our present and previous studies (Azuma et al. 2012). This difference of T cell activation may be attributable to the fact that polyI:C consists of a variety of length of polyI chains and polyC chains with a lot-to-lot heterogeneity. In addition, the amounts of Ags in Azuma's experiment are higher than those in the present experiment (Azuma et al. 2012). CD40 stimulation by specific Ab results in high enhancement of cross-priming of CD8 T cells (Charalambous et al. 2006) and CD40 was up-regulated in CD8α+ DCs by polyI:C treatment, but the CD40

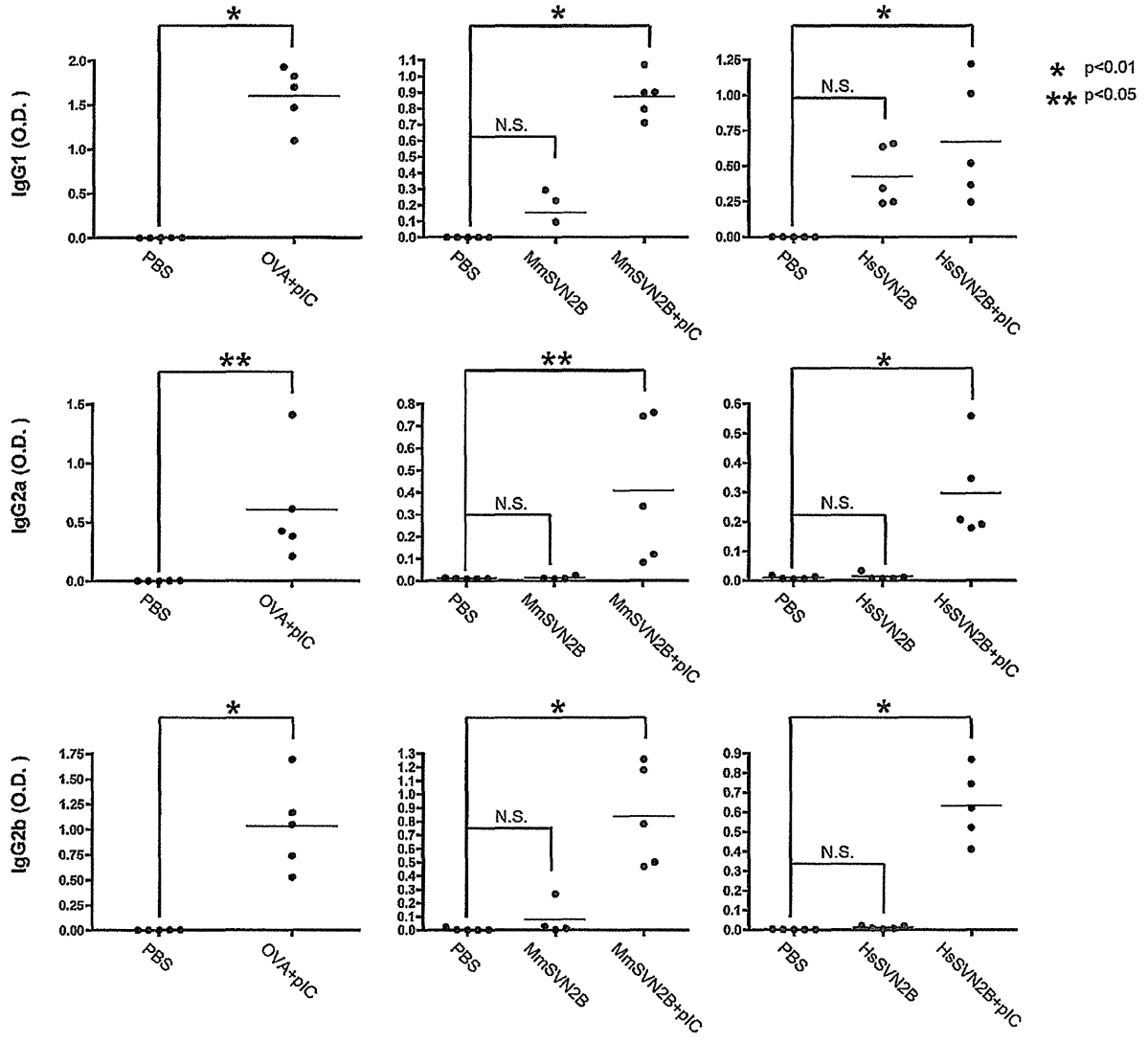

Fig. 5. Production of OVA and SVN-specific antibodies. Sera were collected from immunized mice at once a week for 4 weeks. Anti-OVA or anti-SVN in sera was assessed by ELISA using antiserum for IgG2a/b and IgG1. $^{*}p < 0.01$, $^{**}p < 0.05$.

levels were also variable depending upon the polyI:C lots. Development of a synthesizing method for defined length of RNA duplex will settle the issue.

Two points are noted. First, polyI:C, an RNA adjuvant, induces $CD4^+$ T cells in addition to the reported cases of $CD8^+$ T cells. The factors that participate in polyI:C-mediated CD4+ T cell proliferation and the kind of $CD4^+$ T subsets that are predominantly induced by polyI:C remain unknown. PolyI:C is primarily a potential activator of the IFN-inducing pathways RIG-I/MDA5 and TLR3 (Matsumoto & Seya 2008). These pathways allow host immune cells to produce type I/III IFNs and cytokines and are soluble effectors against cancer. TLR3 preferentially induces cross-presentation in $CD8\alpha^+$ DC in response to dsRNA including polyI:C (Schulz et al., 2005; Azuma et al. 2012) and causes proliferation of $CD8^+$ T cells including cells that respond to TAAs *via* cross-priming (Azuma et al. 2012). $CD4^+$ T cells that are likely evoked by polyI:C stimulation function in antitumor immunity since their helper function is usually suppressed in tumor-bearing mice and can be relieved by innate immune response (Lee et al. 2013). Stimulation with polyI:C + SVN Ag might change a tumor-derived suppressive environment to an environment suitable for primary activation and maintenance of Ag-specific cytotoxic $CD8^+$ T cell responses (Ridge et al. 1998; Janssen et al. 2003).

According to a recent report, however, adoptively transferred $CD4^+$ T cells induce tumor rejection independently of $CD8^+$ T cells (Corthay et al. 2005; Perez-Diez et al. 2007). This rejection is apparently based on cytokines released from $CD4^+$ T cells (Corthay et al. 2005) and on interaction with $CD4^+$ T cells and other immune cells such as macrophages (Mfs) and natural killer (NK) cells (Perez-Diez et al. 2007). DCs stimulated with polyI:C also result in NK cell activation after DC-NK cell-to-cell contact (Akazawa et al. 2007). Mfs in tumors might be a direct target of dsRNA, which converts tumor-supporting Mfs into tumoricidal Mfs (Shime et al. 2012). IL-12p40 is preferentially produced *via* the TICAM-1/Batf3 pathway in response to dsRNA (Azuma et al. 2013). Thus, a variety of cellular effectors can be triggered as antitumor agents by administration of dsRNA with TAA peptides or proteins. We found that $CD4^+$ T cells with Th1 properties were effectors induced by polyI:C possibly acting as an antitumor agent in SVN-responding tumor cells. Although epitope sequence and hydrophobicity might affect Th1 polarization in mice, $CD4^+$ T effectors are successfully induced in tumor-bearing or tumor-implanted mice by stimulation with MmSVN2B + polyI:C.

Hence, *in vivo* administration of an RNA adjuvant with Ag proteins induce $CD4^+$ helper T cells secondary to class II presentation in DCs, together with induction of type I IFNs and cytokines. $CD4^+$ T cells also facilitate Ab production caused by stimulation of B cell development (Mak et al. 2003).

Notably, this is a specific feature of RNA adjuvants, since TLR2 agonist Pam2 lipopeptides such as Pam2CSK4 and MALP2s induce antitumor CTLs with sufficient potential (Chua et al. 2014) but fail to induce DC-mediated antitumor NK cell activation (Yamazaki et al. 2011; Sawahata et al. 2011). $CD4^+$ T cells with regulatory modes such as Tregs and Tr-1 cells and IL-10 were induced by Pam2 peptide in the presence of Ag (Yamazaki et al. 2011). Nevertheless, robust proliferation of antitumor CTL is induced by Pam2 lipopeptides (Chua et al. 2014). Thus, the mode of $CD8^+$ T cell proliferation is differentially modulated between TLR2 and TLR3/MDA5 agonists.

The other point is how self-Ag-reactive $CD4^+$ T cells that act as Th1 effectors in SVN-based immunotherapy are generated. Proliferation of self-reactive T cells is prevented in normal mice, so the levels of self-reactive T cells are usually lower than the detection limit of assays (Gebe et al. 2003). Self-reactive $CD4^+$ T cells might be positively regulated by polyI:C in the presence of protein antigen, since mice, when exposed to DNA/RNA, harbor autoimmune diseases against the protein (Mills 2011). However, even with Ag proteins, polyI:C induced minimal cross-priming of $CD8^+$ T cells in our setting, as with previous reports (Charalambous et al. 2006). In this and other studies, both syngeneic and xenogeneic CD4 epitopes prime $CD4^+$ T cells, stimulating Ab production and Th1 polarization with antitumor activity, but with little association with CTL induction (Charalambous et al. 2006). Our SVN results suggested that self-responsive CD4 epitopes that are identical in sequence in human and mouse SVN have a conserved function as a Th1 skewer, albeit modest, in mice by stimulating DCs and Mfs to prime T and B cells. In this context, however, a question remains to be settled about why the insertion of the 2B sequence in MmSVN caused induction of auto-reactive $CD4^+$ T cells secondary to the class II presentation of the common SVN sequence (53–67) rather than the reported uncommon 13–27 region.

Generally, the presence of Tregs and regulatory cytokines such as IL-10 usually suppresses the function of self-reactive $CD4^+$ T effectors, so an autoimmune response cannot be detected (Danke et al. 2004; Quezada et al. 2010). In tumor-bearing mice, polyI:C releases the restriction of T cell autoreactivity by Tregs to enhance $CD4^+$ T function in a tumor microenvironment. Although the level of Treg cells increases in MALP2s-stimulated tumor-bearing mice (Yamazaki et al. 2011), the amount of Treg cells is not affected by polyI:C injection (Chua et al. 2014). Signs of autoimmune diseases have not yet been observed in mice that received intermittent administration of polyI:C under our conditions. Further studies on the function of regulatory factors in tumor-bearing mice after treatment with various adjuvants are needed to determine the balance between $CD4^+$ T effector functions and regulatory factors including Tregs (Quezada et al. 2010; Corthay et al. 2005).

It has been reported that treatment of murine glioma with DCs loading MmSVN long overlapping peptide covering CD4 and CD8 epitopes (DC therapy) conferred good prognosis on tumor-bearing mice (Ciesielski et al., 2008). In previous trials on peptide vaccine therapy, SVN2B peptide + IFN-α resulted in clinical improvements and enhanced immunological responses of patients (Kameshima et al. 2013). Treatment with SVN2B peptide alone did not result in good prognosis or effective tumor regression in late stage patients with cancer, however (Tsuruma et al. 2008; Honma et al. 2009). These results suggest that both killer and helper T cells are required for *in vivo* induction of tumor regression, as previously suggested (Perez-Diez et al. 2007). NK cells, Mfs, and soluble and angiogenetic factors might be involved in tumor rejection (Shime et al. 2012; Müller-Hermelink et al., 2008; Coussens and Werb 2002) in addition to Ag + polyI:C. According to the study with Ag and polyI:C, a protein or long peptide Ag containing CD4 epitopes, adjuvant RNA and additional factors that disable immunoregulatory factors, are required to effectively induce TAA-specific killer and helper T cell proliferation and subsequent tumoricidal activity in future studies (Casares et al. 2001). Ag peptides should be designed to present both class I and class II peptides on DCs to facilitate proliferation of $CD4^+$ T cells and Ab production. Methods for inducing potential $CD8^+$ CTLs against tumors still need to be considered.

Competing interests

The authors have declared that no competing interest exists.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.imbio.2014.08.017.

References

Ahonen, C.L., Doxsee, C.L., McGurran, S.M., Riter, T.R., Wade, W.F., Barth, R.J., Vasilakos, J.P., Noelle, R.J., Kedl, R.M., 2004. Combined TLR and CD40 triggering induces potent CD8+ T cell expansion with variable dependence on type I IFN. J. Exp. Med. 199, 775–784.

Altieri, D.C., 2001. The molecular basis and potential role of survivin in cancer diagnosis and therapy. Trends Mol. Med. 7, 542–547.

Akazawa, T., Ebihara, T., Okuno, M., Okuda, Y., Shingai, M., Tsujimura, K., Takahashi, T., Ikawa, M., Okabe, M., Inoue, N., Okamoto-Tanaka, M., Ishizaki, H., Miyoshi, J., Matsumoto, M., Seya, T., 2007. Antitumor NK activation induced by the Toll-like receptor 3-TICAM-1 (TRIF) pathway in myeloid dendritic cells. Proc. Natl. Acad. Sci. U. S. A. 104, 252–257.

Ambrosini, G., Adida, C., Altieri, D.C., 1997. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat. Med. 3, 917–921.

Andersen, M.H., Pedersen, L.O., Becker, J.C., Straten, P.T., 2001. Identification of a cytotoxic T lymphocyte response to the apoptosis inhibitor protein survivin in cancer patients. Cancer Res. 61, 869–872.

Azuma, M., Ebihara, T., Oshiumi, H., Matsumoto, M., Seya, T., 2012. Cross-priming for antitumor CTL induced by soluble Ag + polyI:C depends on the TICAM-1 pathway in mouse CD11c(+)/CD8α(+) dendritic cells. Oncoimmunology 1, 581–592.

Azuma, M., Matsumoto, M., Seya, T., 2013. PolyI:C-derived dendritic cell maturation and cellular effectors depend on TICAM-1-Batf3 axis in mice. Proc. Jpn. Cancer Assoc. 72, 126.

Bevan, M.J., 1976. Cross-priming for a secondary cytotoxic response to minor H antigens with H-2 congenic cells which do not cross-react in the cytotoxic assay. J. Exp. Med. 143, 1283–1288.

Casares, N., Lasarte, J.J., de Cerio, A.L., Sarobe, P., Ruiz, M., Melero, I., Prieto, J., Borrás-Cuesta, F., 2001. Immunization with a tumor-associated CTL epitope plus a tumor-related or unrelated Th1 helper peptide elicits protective CTL immunity. Eur. J. Immunol. 31, 1780–1789.

Charalambous, A., Oks, M., Nchinda, G., Yamazaki, S., Steinman, R.M., 2006. Dendritic cell targeting of survivin protein in a xenogeneic form elicits strong CD4+ T cell immunity to mouse survivin. J. Immunol. 177, 8410–8421, 44.

Chua, B.Y., Olson, M.R., Bedoui, S., Sekiya, T., Wong, C.Y., Turner, S.J., Jackson, D.C., 2014. The use of a TLR2 agonist-based adjuvant for enhancing effector and memory CD8 T-cell responses. Immunol. Cell Biol., http://dx.doi.org/10.1038/icb.2013.102.

Ciesielski, M.J., Kozbor, D., Castanaro, C.A., Barone, T.A., Fenstermaker, R.A., 2008. Therapeutic effect of a T helper cell supported CTL response induced by a survivin peptide vaccine against murine cerebral glioma. Cancer Immunol. Immunother. 57, 1827–1835.

Corthay, A., Skovseth, D.K., Lundin, K.U., Rosjo, E., Omholt, H., Hofgaad, P.O., Haraldsen, G., Bogen, B., 2005. Primary antitumor immune response mediated by CD4+ T cells. Immunity 22, 371–383.
Coussens, L.M., Werb, Z., 2002. Inflammation and cancer. Nature 420, 860–867.
Danke, N.A., Koelle, D.M., Yee, C., Beheray, S., Kwok, W.W., 2004. Autoreactive T cells in healthy individuals. J. Immunol. 172, 5967–6572.
Fukuda, S., Pelus, L.M., 2006. Survivin, a cancer target with an emerging role in normal adult tissues. Mol. Cancer Ther. 5, 1087–1098.
Gebe, J.A., Falk, B.A., Rock, K.A., Kochik, S.A., Heninger, A.K., Reijonen, H., Kwok, W.W., Nepom, G.T., 2003. Low-avidity recognition by CD4+ T cells directed to self-antigens. Eur. J. Immunol. 33, 1409–1417.
Gotoh, M., Takasu, H., Harada, K., Yamaoka, T., 2002. Development of HLA-A2402/K^b transgenic mice. Int. J. Cancer 100, 565–570.
Hirohashi, Y., Torigoe, T., Maeda, A., Nabeta, Y., Kamiguchi, K., Sato, T., Yoda, J., Ikeda, H., Hirata, K., Yamanaka, N., Sato, N., 2002. An HLA-A24-restricted cytotoxic T lymphocyte epitope of a tumor-associated protein, survivin. Clin. Cancer Res. 8, 1731–1739.
Honma, I., Kitamura, H., Torigoe, T., Takahashi, A., Tanaka, T., Sato, E., Hirohashi, Y., Masumori, N., Tsukamoto, T., Sato, N., 2009. Phase I clinical study of anti-apoptosis protein survivin-derived peptide vaccination for patients with advanced or recurrent urothelial cancer. Cancer Immunol. Immunother. 58, 1801–1807.
Idenoue, S., Hirohashi, Y., Torigoe, T., Sato, Y., Tamura, Y., Hariu, H., Yamamoto, M., Kurotaki, T., Tsuruma, T., Asanuma, H., Kanaseki, T., Ikeda, H., Kashiwagi, K., Okazaki, M., Sasaki, K., Sato, T., Ohmura, T., Hata, F., Yamaguchi, K., Hirata, K., Sato, N., 2005. A potent immunogenic general cancer vaccine that targets survivin, an inhibitor of apoptosis proteins. Clin. Cancer Res. 11, 1474–1482.
Janssen, E.M., Lemmens, E.E., Wolfe, T., Christen, U., von Herrath, M.G., et al., 2003. CD4+ T cells are required for secondary expansion and memory in CD8+ T lymphocytes. Nature 421, 852–856.
Kameshima, H., Tsuruma, T., Kutomi, G., Shima, H., Iwayama, Y., Kimura, Y., Imamura, M., Torigoe, T., Takahashi, A., Hirohashi, Y., Tamura, Y., Tsukahara, T., Kanaseki, T., Sato, N., Hirata, K., 2013. Immunotherapeutic benefit of α-interferon (IFNα) in survivin2B-derived peptide vaccination for advanced pancreatic cancer patients. Cancer Sci. 104, 124–129.
Kobayashi, K., Hatano, M., Otaki, M., Ogasawara, T., Tokuhisa, T., 1999. Expression of a murine homologue of the inhibitor of apoptosis protein is related to cell proliferation. Proc. Natl. Acad. Sci. U. S. A. 96, 1457–1462.
Lee, M.K.4th, Xu, S., Fitzpatrick, E.H., Sharma, A., Graves, H.L., Czerniecki, B.J., 2013. Inhibition of CD4+CD25+ regulatory T cell function and conversion into Th1-like effectors by a Toll-like receptor-activated dendritic cell vaccine. PLOS ONE 8, e74698.
Li, F., 2005. Role of survivin and its splice variants in tumorigenesis. Br. J. Cancer 92, 212–216.
Mahotka, C., Wenzel, M., Springer, E., Gabbert, H.E., Gerharz, C.D., 1999. Survivin-deltaEx3 and survivin-2B: two novel splice variants of the apoptosis inhibitor survivin with different antiapoptotic properties. Cancer Res. 59, 6097–6102.
Mahotka, C., Liebmann, J., Wenzel, M., Suschek, C.V., Schmitt, M., Gabbert, H.E., Gerharz, C.D., 2002. Differential subcellular localization of functionally divergent surviving splice variants. Cell Death Differ. 9, 1334–1342.
Mak, T.W., Shahinian, A., Yoshinaga, S.K., Wakeham, A., Boucher, L.M., Pintilie, M., Duncan, G., Gajewska, B.U., Gronski, M., Eriksson, U., Odermatt, B., Ho, A., Bouchard, D., Whorisky, J.S., Jordana, M., Ohashi, P.S., Pawson, T., Bladt, F., Tafuri, A., 2003. Costimulation through the inducible costimulator ligand is essential for both T helper and B cell functions in T cell-dependent B cell responses. Nat. Immunol. 4, 765–772.
Matsumoto, M., Seya, T., 2008. TLR3: interferon induction by double-stranded RNA including poly(I:C). Adv. Drug Deliv. Rev. 60, 805–812.
Mills, K.H., 2011. TLR-dependent T cell activation in autoimmunity. Nat. Rev. Immunol. 11, 807–822.
Müller-Hermelink, N., Braumüller, H., Pichler, B., Wieder, T., Mailhammer, R., Schaak, K., Ghoreschi, K., Yazdi, A., Haubner, R., Sander, C.A., Mocikat, R., Schwaiger, M., Förster, I., Huss, R., Weber, W.A., Kneilling, M., Röcken, M., 2008. TNFR1 signaling and IFN-gamma signaling determine whether T cells induce tumor dormancy or promote multistage carcinogenesis. Cancer Cell 13, 507–518.
Nishiguchi, M., Matsumoto, M., Takao, T., Hoshino, M., Shimonishi, Y., Tsuji, S., Begum, N.A., Takeuchi, O., Akira, S., Toyoshima, K., Seya, T., 2001. Mycoplasma *fermentans* lipoprotein M161Ag-induced cell activation is mediated by Toll-like receptor 2: role of N-terminal hydrophobic portion in its multiple functions. J. Immunol. 166, 2610–2616.
Okada, H., Bakal, C., Shahinian, A., Elia, A., Wakeham, A., Suh, W.K., Duncan, G.S., Ciofani, M., Rottapel, R., Zuniga-Pflucker, J.C., Mak, T.W., 2004. Survivin loss in thymocytes triggers p53-mediated growth arrest and p53-independent cell death. J. Exp. Med. 199, 399–410.
Osen, W., Soltek, S., Song, M., Leuchs, B., Steitz, J., Tüting, T., Eichmüller, S.B., Nguyen, X.D., Schadendorf, D., Paschen, A., 2010. Screening of human tumor antigens for CD4T cell epitopes by combination of HLA-transgenic mice, recombinant adenovirus and antigen peptide libraries. PLOS ONE 5, e14137.
Perez-Diez, A., Joncker, N.T., Choi, K., Chan, W.F., Anderson, C.C., Lantz, O., Matzinger, P., 2007. CD4 cells can be more efficient at tumor rejection than CD8 cells. Blood 109, 5346–5354.
Quezada, S.A., Simpson, T.R., Peggs, K.S., Merghoub, T., Vider, J., Fan, X., Blasberg, R., Yagita, H., Muranski, P., Antony, P.A., Restifo, N.P., Allison, J.P., 2010. Tumor-reactive CD4(+) T cells develop cytotoxic activity and eradicate large established melanoma after transfer into lymphopenic hosts. J. Exp. Med. 207, 637–650.
Ridge, J.P., Di Rosa, F., Matzinger, P., 1998. A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. Nature 393, 474–478.
Rosenberg, S.A., Yang, J.C., Restifo, N.P., 2004. Cancer immunotherapy: moving beyond current vaccines. Nat. Med. 10, 909–915.
Sawahata, R., Shime, H., Yamazaki, S., Inoue, N., Akazawa, T., Fujimoto, Y., Fukase, K., Matsumoto, M., Seya, T., 2011. Failure of mycoplasma lipoprotein MALP-2 to induce NK cell activation through dendritic cell TLR2. Microbes Infect. 13, 350–358.
Schmitz, M., Diestelkoetter, P., Weigle, B., Schmachtenberg, F., Stevanovic, S., Ockert, D., Rammensee, H.G., Rieber, E.P., 2000. Generation of survivin-specific CD8+ T effector cells by dendritic cells pulsed with protein or selected peptides. Cancer Res. 60, 4845–4849.
Schulz, O., Diebold, S.S., Chen, M., Näslund, T.I., Nolte, M.A., Alexopoulou, L., Azuma, Y.T., Flavell, R.A., Liljeström, P., Reis e Sousa, C., 2005. Toll-like receptor 3 promotes cross-priming to virus-infected cells. Nature 433, 887–892.
Seya, T., Matsumoto, M., 2009. The extrinsic RNA-sensing pathway for adjuvant immunotherapy of cancer. Cancer Immunol. Immunother. 58, 1175–1184.
Seya, T., Azuma, M., Matsumoto, M., 2013. Targeting TLR3 with no RIG-I/MDA5 activation is effective in immunotherapy for cancer. Expert Opin. Ther. Targets 17, 533–544.
Shime, H., Matsumoto, M., Oshiumi, H., Tanaka, S., Nakane, A., Iwakura, Y., Tahara, H., Inoue, N., Seya, T., 2012. Toll-like receptor 3 signaling converts tumor-supporting myeloid cells to tumoricidal effectors. Proc. Natl. Acad. Sci. U. S. A. 109, 2066–2071.
Topalian, S.L., Gonzales, M.I., Parkhurst, M., Li, Y.F., Southwood, S., Sette, A., Rosenberg, S.A., Robbins, P.F., 1996. Melanoma-specific CD4+ T cells recognize nonmutated HLA-DR-restricted tyrosinase epitopes. J. Exp. Med. 183, 1965–1971.
Tsuruma, T., Iwayama, Y., Ohmura, T., Katsuramaki, T., Hata, F., Furuhata, T., Yamaguchi, K., Kimura, Y., Torigoe, T., Toyota, N., Yagihashi, A., Hirohashi, Y., Asanuma, H., Shimozawa, K., Okazaki, M., Mizushima, Y., Nomura, N., Sato, N., Hirata, K., 2008. Clinical and immunological evaluation of anti-apoptosis protein, survivin-derived peptide vaccine in phase I clinical study for patients with advanced or recurrent breast cancer. J. Transl. Med. 6, 24.
Yamazaki, S., Okada, K., Maruyama, A., Matsumoto, M., Yagita, H., Seya, T., 2011. TLR2-dependent induction of IL-10 and Foxp3+ CD25+ CD4+ regulatory T cells prevents effective anti-tumor immunity induced by Pam2 lipopeptides in vivo. PLOS ONE 6, e18833.

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REVIEW ARTICLE

Trials of vaccines for pancreatic ductal adenocarcinoma: Is there any hope of an improved prognosis?

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Abstract Pancreatic tumors are chemoresistant and malignant, and there are very few therapeutic options for pancreatic cancer, as the disease is normally diagnosed at an advanced stage. Although attempts have been made to develop vaccine therapies for pancreatic cancer for a couple of decades, none of the resultant protocols or regimens have succeeded in improving the clinical outcomes of patients. We herein review vaccines tested within the past few years, including peptide, biological and multiple vaccines, and describe the three sets of criteria used to evaluate the therapeutic activity of vaccines in solid tumors.

Keywords Pancreatic cancer · Vaccine · Immunomodulation

Introduction

Pancreatic cancer is the fourth leading cause of cancer-related death in the United States [1–3] and the fifth most common cause of such deaths in Japan [4]. Although surgical resection is considered to be the only curative therapy for pancreatic cancer, only 20 % of patients have resectable disease at the time of diagnosis [5, 6]. In addition, advanced pancreatic cancer patients exhibit a median survival time (MST) of approximately six months and a 5-year overall survival rate of less than 5 %, despite efforts to manage the tumors with chemotherapy, radiotherapy and other treatments [3, 5–8].

In 1997, Burris et al. reported that gemcitabine monotherapy is superior to fluorouracil (5-FU) monotherapy for

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Table 1 Chemotherapy for advanced pancreatic cancer

	Median survival time (months)	Overall response rate (%)	Trial name	References
Gemcitabine	5.65	5.4		J Clin Oncol 1997;15: 2403–13.
Gemcitabine + erlotinib	6.24	8.6	NCIC CTG PA.3	J Clin Oncol 2007;25: 1960–6.
FOLFIRINOX	11.1	31.6	ACCORD 11	N Engl J Med 2011;364: 1817–25.
Nab-paclitaxel + gemcitabine	8.7	29.2	MPACT trial NCT00844649	N Engl J Med 2013;369: 1691–703.
Gemcitabine +TS-1	10.1	29.3	GEST trial	J Clin Oncol 2013; 31:640–8.

treating pancreatic ductal adenocarcinoma (PDAC) [9]. Gemcitabine monotherapy has subsequently become the standard chemotherapy for PDAC, resulting in an MST of 5.65 months (Table 1). Currently, three protocols have proven to be superior to gemcitabine monotherapy. Combining gemcitabine with erlotinib improved the MST of PDAC to 6.24 months in the NCIC CTG PA3 trial [10], while combining gemcitabine with nab-paclitaxel improved the MST to 8.7 months in the MPACT trial [11]. FOLFIRINOX achieved the longest MST for PDAC (11.1 months) in the ACCORD11 trial [12], and the GEST study obtained similar clinical outcomes. S-1 is an oral fluoropyrimidine derivative that has been shown to be effective against various cancers, and a previous study found that it is at least as effective as gemcitabine against PDAC [13]. In addition, treatment with a combination of gemcitabine + S-1 has been demonstrated to result in an MST of 10.1 months [14]. Although these chemotherapies extend the survival period among PDAC patients, they also result in serious adverse events. Therefore, the optimal chemotherapy regimen for PDAC depends on the patient's performance status.

There have been numerous attempts to develop vaccine therapies for cancer over the past century [2, 3]. Although clinical trials of such vaccines have obtained promising results in specific patients, none of the tested vaccines has exhibited significant improvements in efficacy compared with established therapies. In addition, several issues must be resolved before vaccine therapies can be used in the clinical setting. Tumor-associated antigens (TAA) have been demonstrated to recognize specific human leukocyte antigens (HLA) [15]. Theoretically, the tumor lysate contains all of the antigens expressed by the tumor, and cytotoxic T lymphocytes (CTL) are capable of recognizing some of these antigens [16]. All vaccines for pancreatic cancer are based on the fact that CTL recognize TAA expressed on tumor cells and subsequently attack these cells. The question is how strongly and specifically each TAA stimulates CTL in vivo in the clinical setting. Immune tolerance can develop via various mechanisms, including the downregulation of the major histocompatibility complex (MHC) molecule expression, induction of T cell anergy, reductions in the number of immune effectors and increases in the number of regulatory T cells [17, 18], which may explain why no cancer vaccine therapy has been established as a standard treatment for advanced PDAC. Therefore, in this study, we comprehensively reviewed the clinical outcomes of vaccine therapy against advanced PDAC.

Peptide-based vaccines developed within the past few years

MUC1

Mucin 1, cell surface associated, (MUC1) is a type I transmembrane protein containing multiple tandem repeats of a 20-amino acid sequence. Several MUC1 peptides have been tested as vaccines in the clinical setting; however, most of them have failed to activate CTL [19–21]. Ramanathan et al. [22]; Yamamoto et al. [23] injected pancreatic patients with a vaccine containing a 100-mer extracellular tandem repeat domain of MUC1 and Montanide ISA-51, and both studies obtained similar clinical responses; i.e., the authors detected cytokines (interferon (IFN)-γ or interleukin (IL)-4) and anti-MUC1 antibodies in the patients' sera but did not observe any significant clinical effects. Another recent study involving a vaccine based on a different MUC1 epitope showed similar clinical outcomes, i.e., all seven patients had progressive disease (PD), although some of the patients exhibited immunological responses, such as IFN-γ and granzyme B secretion [24].

K-RAS mutants

K-RAS mutations are frequently found in patients with PDAC. Vaccines targeting mutations in codon 12 of the K-RAS gene have been tested as treatments for advanced [25] or postoperative [26] PDAC in the clinical setting. Gjertsen et al. [[21]] investigated the utility of a K-RAS peptide vaccine containing granulocyte–macrophage colony-stimulating factor (GM-CSF) in 10 patients who had undergone potentially curative

resection (CTN RAS 95002) and 38 patients with advanced disease (CTN RAS 97004). In that study, one patient achieved a partial response (PR), which lasted for 28 months, and the MST of the immunological responders was 4.9 months, compared to 2.0 months for the non-responders.

Human telomerase reverse transcriptase (hTERT)

Human telomerase reverse transcriptase (hTERT) is frequently expressed in cancer cells [27]. hTERT maintains functional telomeres at the end of chromosomes, which protect against cell senescence [28]. A vaccine against pancreatic cancer containing the telomerase peptide GV1001: hTERT (611-626) and GM-CSF was examined by Bernhardt et al. [29], who found the MST of the immunological responders and non-responders to be 7.2 and 2.9 months, respectively.

Vascular endothelial growth factor receptor 2 (VEGFR2)

Vascular endothelial growth factor (VEGF) plays an important role in the progression of PDAC. The type 2 VEGF receptor (VEGFR2) is expressed in PDAC and associated with tumor neovascularization. Miyazawa et al. [[30]] investigated the efficacy of combined treatment consisting of PDAC with a VEGFR2-169 peptide-based vaccine and gemcitabine chemotherapy and reported that one patient achieved a PR, while the disease control rate was 67 %. In addition, the MST was 7.7 months, although 15/18 patients were chemotherapy naive.

G17DT (gastrimmune)

Gastrin is expressed in PDAC and plays a role in regulating the autocrine, paracrine and endocrine systems [31]. The administration of the anti-gastrin immunogen G17DT results in increased serum antibody levels and reduced tumor growth in patients with gastrointestinal malignancies [32]. A randomized, double-blind, placebo-controlled multicenter trial of G17DT was also recently performed [33]. Although, among the intention to treat (ITT) population, no significant differences in MST were detected between the PDAC patients treated with G17DT and those given the placebo, the MST of the two groups differed significantly after excluding major protocol violators and censoring for chemotherapy.

Heat shock protein (HSP)

Heat shock protein (HSP) itself is not an immunogen; however, it acts as a chaperone or carrier of antigenic peptides and possesses a repertoire of cellular peptides for pancreatic cancer [34]. Furthermore, HSPPC-96 (Oncophage) has been tested as a vaccine in the adjuvant setting after complete resection of PDAC [35]. In the latter study, the MST of PDAC was reported to be 2.9 months after surgery; however, this did not result in further clinical studies because only two of 10 patients exhibited increased enzyme-linked immunospot (ELISPOT) reactivity.

Biological vaccines

Fowlpox viral vaccine

Carcinoembryonic antigen (CEA) and MUC1 are highly expressed in PDAC [36]. Viral vectors carrying CEA, MUC1 and TRICOM [a triad of costimulatory molecules: B7.1, intercellular adhesion molecule 1 (ICAM-1) and lymphocyte function-associated antigen 3 (LFA-3)] have been investigated as vaccines against advanced PDAC [37]. In one study, a vaccinia viral vector was used for the initial T cell priming, and a fowlpox viral vector was used for immune boosting. Although this treatment resulted in an MST of 6.3 months (1.5–21.1 months), the five patients who showed T cell responses achieved a longer survival period than the five patients who did not (15.1 and 3.9 months, respectively; $P = 0.002$) [38]. It should be noted that GM-CSF was used as a vaccine adjuvant in the latter trial (Table 2).

Live-attenuated, double-deleted (LADD) Listeria monocytogene vaccine

ANZ-100 is a live-attenuated double-deleted Listeria monocytogene strain (LADD; Lm ΔactA/ΔinlB) found to induce a local proinflammatory response, resulting in the activation of innate and adaptive effector cells [39]. Mesothelin is expressed in PDAC and plays an important role in tumor progression [40]. CRS-207 is a LADD Lm strain that delivers mesothelin antigens into class I and II antigen-processing pathways [41]. In a study examining the utility of CRS-207 as a treatment for advanced cancer, three of the seven subjects with PDAC were long-term survivors, although the detection of a mesothelin-specific T cell response was not correlated with survival [41].

Recent vaccine therapies

WT1

Kobayashi et al. reported a retrospective analysis of 255 advanced PDAC patients who were treated with dendritic

Table 2 Peptide-based vaccines and biological vaccines for advanced pancreatic cancer

Author	Journal	Antigen peptide	Sequences	Combination	Patients	Outcome/MST
Yamamoto	Anticancer Res. 2005;25:3575–9	MUC1	10-mer extracellular tandem repeat domain: $(GVTSAPDTRPAPGSTAPPAH)_5$	Montanide ISA-51	6	1/6 SD
Rong	Clin Exp Med. 2012;12:173–80	MUC1	PDTRPAPGSTAPPAHGVTSA	DC cells	7	All PD
Gjertsen	Int J Cancer. 2001;92:441–50	K-ras	KLVVVGAGGVGKSALTI Asp: D Arg: R Val: V Cyc: C	GM-CSF	38	1 PR 10 SD (10.2 M; 3-23 M) 27 PD 4.9 M responders 2.0 M non-responders
Abou-Alfa	Am J Clin Oncol. 2011;34:321–5	ras12R ras12 V ras12D Wild-type ras	TEYKLVWGARGVGKSALTIQ TEYKLVWGAVGVGKSALTIQ TEYKLVWGADGVGKSALTIQ TEYKLVWGAGGVGKSALTIQ	hGM-CSF	24	Postoperative adjuvant treatment
Bernhardt	Br J Cancer. 2006;95:1474–82	Telomerase hTERT (611–626)	GV1001; EARPALLTSRLRFIPK	GM-CSF	38	7.2 M (24 responders) 2.9 M (14 non-responders)
Miyazawa	Cancer Sci. 2010;101:433–9	VEGFR2-169	RFVPDGNRI	Gemcitabine	18	7.7 M
Gilliam	Pancreas. 2012;41:374–9	Anti-gastrin G17DT Gastrimmune	EGPWLEEEEEAYGWMDF-DT (diphtheria toxoid)	G17DT vs. placebo	152	5.0 M vs 2.8 M
Maki	Dig Dis Sci. 2007;52:1964–72	HSP HSPPC-96 (gp96, Oncophage)			10	Postoperative adjuvant treatment 2.7 Y
Kaufman	J Transl Med. 2007;5:60	MUC1 and CEA	CEA agonist peptide CAP1-6D (YLSGADLNL) MUC-1 agonist peptide P-93L (ALWGQDVTSV)	B7.1, ICAM-1, LFA-3 (TRICOM) Vaccinia virus: PANVAC-V Fowlpox virus: PANVAC-F GM-CSF	10	6.3 M
Le	Clin Cancer Res. 2012;18:858–68	Listeria vaccine ANZ-100, CRS-207			9 vs. 17	NA

Table 3 Recently developed peptide-based vaccines and multiple vaccines for advanced pancreatic cancer

Author	Journal	Antigen peptide	Sequences	Restricted HLA	Combination	Patients	Outcome/MST
Kobayashi	Cancer Immunol Immunother. 2014;63:797–806	WT1 MUC1	CYTWNQMNL RMFPNAPYL TRPAPGSTAPPAHG-VTSAP DTRPAPGSTAP	A24:02 A02:01/02:06 Any A	DC cells OK432	255	9.9 M 10.4 M (erythema)
Nishida	J Immunother. 2014;37:105–14	WT1	CYTWNQMNL	A24:02	Weekly 1000 mg/m^2 GEM	31	8.1 M 10.9 M (DTH)
Asahara	J Translation Res. 2013;11:291	KIF20A-66	KVYLRVRPLL	A2402	Montanide ISA51VG	31	4.7 M 6.1 M (reaction)
Suzuki	J Immunother. 2014;37:36–42	KIF20A-10-66	KVYLRVRPLL	A2402	Montanide ISA51VG	9	5.8 M
Geynisman	J ImmunoThera Cancer. 2013;1:8	CEA CAP1-6D	YLSGADLNL	A2	Montanide/GM-CSF	19	11.1 M
Kameshima	Cancer Sci. 2013;104:124–9	SVN2B	AYACNTSTL	A2402	Montanide/IFN-oc	6	(9.6 M)
Yutani	Oncology Reports. 2013;30:1094–100	31 vaccine peptides		A2, A24, A3, A26	Mono: 8 Chemo: 33	41	7.9 M 9.6 M (chemo)
Kimura	Pancreas. 2012;41:195–205	WT1, Her2, CEA, MUC1, CA125, autologous tumor lysate			DC cells plus LAK plus GEM and S1 OK432	49	S: 8.0 M G: 12.0 M GS + LAK: 16.9 M
Le	J Clin Oncol. 2014;32(suppl 3):Abstract 177	GVAX pancreas and CRS-207 vs. GVAX pancreas alone	Irradiated GM-CSF-secreting allogeneic pancreatic tumor vaccine (GVAX pancreas)		Cyclophosphamide	90	6.1 M vs. 3.9 M 9.7 M (3 or more rounds of vaccine therapy)

cell (DC) vaccines containing Wilms tumor 1 (WT1) and MUC1 after being recruited from seven institutions that followed a unified standard operating procedure. The MST of these patients was 9.9 months [42]. Nishida et al. also examined the utility of chemo-vaccine therapy in which a WT1-based vaccine was used in combination with the administration of 1,000 mg/m^2 of gemcitabine weekly. The latter regimen resulted in an MST of 8.1 months among 31 advanced PDAC patients [43]. In addition, the MST of the immunological responders in these two studies was very similar (10.4 and 10.9 months, respectively) (Table 3).

KIF20A

Kinesin family member 20A (KIF20A) plays an important role in the trafficking of molecules and organelles [44] and is one of the molecules targeted by vaccines against PDAC. A KIF20A vaccine was recently tested using different regimens, including vaccine monotherapy [45] and chemo-vaccine therapy involving gemcitabine [46], and similar MST values were reported in both studies (4.7 and 5.8 months, respectively).

Carcinoembryonic antigen (CEA)

CEA is a 180-kDa immunoglobulin-like molecule expressed on the surface of 90 % of PDAC tumor cells [47]. CAP1-6D, a modified CEA peptide, was combined with Montanide/GM-CSF to produce a vaccine against pancreatic cancer that was subsequently tested in advanced PDAC patients [48]. The MST of the 19 patients was 11.1 months, and one patient, randomized into the 0.01 mg arm, achieved a complete response (CR).

Survivin2B

Survivin is a member of the inhibitors of apoptosis (IAP) family of proteins that protect apoptotic signals by inhibiting the caspase activity [49, 50]. Hence, survivin-expressing cancer cells escape from apoptosis and do not die. Using a peptide-binding assay, we found that the survivin2B 80–88 peptide induces a strong CTL response [51]. We also examined the effects of a survivin2B 80–88 peptide-based vaccine on various cancers in the clinical setting and obtained promising outcomes. In particular, the anti-tumor effect of the survivin2B 80–88 peptide was enhanced by combining it with incomplete Freud's adjuvant and IFN-α injection. Our preliminary clinical study demonstrated a 66.6 % disease control rate in advanced PDAC patients (four of six patients) [52]. Moreover, the PDAC patients in our recent clinical phase I study exhibited an MST of 9.6 months.

Table 4 Evaluation of therapeutic activity in solid tumors

Method	WHO	RECIST	IrRC
	Sum of the products of the two longest perpendicular dimensions (bidimensional)	Sum of the longest dimensions (unidimensional)	Sum of the products of the two longest perpendicular dimensions (SPD) of all index lesions. (bidimensional)
No. of measured lesions	All lesions	Five per organ, 10 in total	Five per organ, 10 in total, and five cutaneous index lesions
CR	Disappearance of all known disease, confirmed at 4 weeks	Disappearance of all known disease, confirmed at 4 weeks	Disappearance of all known disease, confirmed at 4 weeks apart
PR	>50 % decrease in total tumor size, confirmed at 4 weeks	>30 % decrease in total tumor size, confirmed at 4 weeks	>50 % decrease in tumor burden compared with baseline in two observations at least 4 weeks apart
SD	CR, PR, and PD criteria not met	CR, PR, and PD criteria not met	CR, PR, and PD criteria not met
PD	>25 % increase in total tumor size; no CR, PR, or SD documented before increase in tumor size; new lesion (s); > 25 % increase in size of one lesion	>20 % increase in total tumor size; no CR, PR, or SD documented before increase in tumor burden; new lesion (s)	>25 % increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 weeks apart

Tumor burden = $SPD_{index\ lesions}$ +SPD new, measurable lesions

Multiple vaccines

Personalized peptides

In a previous study, a set of 31 peptides was used to create personalized vaccines for advanced PDAC [53]. A maximum of four peptides were selected from among the 31-peptide set based on the results of HLA typing and the patients' peptide-specific IgG titers. Eight patients received vaccine monotherapy, and 31 patients received chemo-vaccine therapy. In the chemo-vaccine therapy group, gemcitabine was administered in eight patients, S-1 was administered in six patients and gemcitabine + S-1 was given in eight patients. The overall MST was 9.6 months, although that of the patients who underwent monotherapy was 7.9 months. Yanagimoto et al. reported similar clinical outcomes for chemo-vaccine therapy involving personalized vaccines and gemcitabine based on the same regimen [54]. The MST of the patients in the latter study was 9.0 months, although that of the immunological responders was 15.5 months. None of the patients in either study achieved CR (Table 3).

Autologous tumor lysate combined with lymphokine-activated killer cell therapy

Kimura et al. treated 49 PDAC patients with vaccines based on five different peptides and autologous tumor lysate, although the vaccine preparation regimens and anti-tumor therapies varied in each case [16]. Two patients achieved CR after treatment with a combination of DC cell and lymphokine-activated killer cell (LAK) therapy. The MST of the patients treated with LAK + gemcitabine and S-1 was 16.9 months, whereas that of all patients was 12.0 months. It should be noted that the survival time was calculated from the day after the first vaccination, which may have resulted in a shorter survival time (by a couple of months) than would have been obtained using the methods employed in other studies. It is very difficult to evaluate the clinical results of this study due to the effects of the different therapeutic strategies used in each case. However, the fact that multiple patients achieved CR will encourage researchers to pursue this approach further.

GVAX pancreas with CRS-207

GVAX is a series of irradiated GM-CSF-secreting allogeneic pancreatic cell lines that elicit broad antigenic responses. CRS-207 is a LADD Lm strain (Lm ΔactA/ΔinlB) that expresses mesothelin and stimulates the innate and adaptive immune systems. A phase II randomized control trial of GVAX pancreas combined with CRS-207 versus GVAX pancreas alone was presented at the 2014 American Society of Clinical Oncology (ASCO) Gastrointestinal Cancers Symposium [55]. Interestingly, the clinical results demonstrated that both treatments had dose-dependent survival benefits. The MST of the patients who received three or more rounds of vaccine therapy was 9.7 months, and the MST of the GVAX with CRS-207 arm was longer than that of the GVAX-alone arm (6.1 vs. 3.9 months; $P = 0.01$) [56].

Evaluation of therapeutic activity in solid tumors

The response of solid tumors is evaluated using either the WHO [57] or RECIST criteria [58]. The RECIST criteria were developed because the WHO criteria are quite complex and measuring all visible lesions in two dimensions is both time consuming and subject to measuring bias [59]. However, the use of immunotherapeutic agents in cancer patients is associated with the following problems: (a) The measurable anti-tumor activity can take longer to appear during immunotherapy than during cytotoxic therapy; (b) Responses to immunotherapy can occur after the standard criteria for progressive disease (PD) have been met; (c) Discontinuing immunotherapy may not be appropriate in some cases, unless PD is confirmed; (d) Allowing for "clinically insignificant" PD (e.g., small new lesions developing in the presence of other responsive lesions) is recommended; and (e) Durable stable disease (SD) may represent the anti-tumor activity [60]. Therefore, the immune-related response criteria (irRC) were developed to evaluate the immunotherapeutic activity in solid tumors [61]. The most important aspects of the irRC criteria are that (a) new lesions are not classified as PD and (b) two consecutive observations obtained at least four weeks apart are required to diagnose PD. However, the clinical utility of the irRC remains unclear and these criteria may require further optimization [61] (Table 4).

Future research topics

Initial time point for survival assessments

The initial time point for survival assessments should be unified to allow clinical outcomes to be compared between studies. Most PDAC patients already have advanced disease at the time of diagnosis [6]. In addition, the adverse effects of chemotherapies differ markedly among the various regimens [8]. Therefore, the status of PDAC patients at the time point at which they are registered can differ both within and between clinical studies. Kobayashi et al. reported that the MST from the date of diagnosis and the MST from the first vaccination are very different (16.5 vs.

9.9 months) [42]. Therefore, MST data must be interpreted carefully.

Vaccine therapy and chemotherapy

The goal of vaccine therapy for cancer is to increase the native immunity of cancer patients. However, chemotherapy causes irreversible damage to proliferating cancer cells as well as immune cells, including T and B cells. Therefore, there is a conflict between the fundamental principles of these two treatments. Chemotherapy is currently the gold standard treatment for advanced PDAC. Although the biological mechanisms of vaccine therapy and chemotherapy conflict with each other, the anti-cancer activity of vaccine monotherapy or chemo-vaccine combination therapy should be greater than that of chemotherapy alone.

Slow clinical response to vaccine therapy

It is very hard to achieve a complete response (CR) with vaccine therapy alone. We reviewed 19 studies involving a total of 860 patients and found that CR responses were obtained in only three cases. Although none of these studies involved a large number of patients, the poor reported response rates are a concern. One of the patients who achieved a CR was administered CEA CAP1-6D + Montanide/GM-CSF therapy, while the other two were treated with WT1, Her2, CEA, MUC1, cancer antigen 125 and autologous tumor lysate vaccines combined with DC cell-based LAK therapy and chemotherapy. Immunological responses require a long time to control tumor growth and achieve remission. The primary goal of vaccine therapy is to achieve long-term SD [62]. Most previous clinical studies of PDAC involved patients with advanced disease for whom no other therapies were available. Therefore, vaccine therapy may be suitable for patients in other clinical stages or possibly a useful postoperative adjuvant therapy. The main advantage of vaccine therapy is that it has few adverse effects, although it has also demonstrated minimal clinical effects in previous trials. We are currently conducting a phase II study of the survivin2B 80–88 peptide + Montanide + IFN-β as a treatment for PDAC (SUCCESS, Study of Unresectable CanCEr with Survivin-2B peptide vaccine in Sapporo: UMIN000012146), in which half of the required patients have been recruited. The clinical results of the SUCCESS phase II study will be reported by the end of next year.

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References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin. 2013;63:11–30.
2. Salman B, Zhou D, Jaffee EM, Edil BH, Zheng L. Vaccine therapy for pancreatic cancer. Oncoimmunology. 2013;2:e26662.
3. Soares KC, Zheng L, Edil B, Jaffee EM. Vaccines for pancreatic cancer. Cancer J. 2012;18:642–52.
4. Hamada T, Nakai Y, Yasunaga H, Isayama H, Matsui H, Takahara N, et al. Prognostic nomogram for nonresectable pancreatic cancer treated with gemcitabine-based chemotherapy. Br J Cancer. 2014;110:1943–9.
5. Saif MW. Controversies in the adjuvant treatment of pancreatic adenocarcinoma. JOP. 2007;8:545–52.
6. Kanda M, Fujii T, Takami H, Suenaga M, Inokawa Y, Yamada S, et al. The combination of the serum carbohydrate antigen 19-9 and carcinoembryonic antigen is a simple and accurate predictor of mortality in pancreatic cancer patients. Surg Today. 2014;44:1692–701.
7. Niccolai E, Prisco D, D'Elios MM, Amedei A. What is recent in pancreatic cancer immunotherapy? Biomed Res Int. 2013;2013:492372.
8. Kitami CE, Kurosaki I, Kawachi Y, Nihei K, Tsuchiya Y, Nomura T, et al. Portal vein infusion chemotherapy with gemcitabine after surgery for pancreatic cancer. Surg Today. 2013;43:33–9.
9. Burris HA 3rd, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol. 1997;15:2403–13.
10. Moore MJ, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, et al. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol. 2007;25:1960–6.
11. Von Hoff DD, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N Engl J Med. 2013;369:1691–703.
12. Conroy T, Desseigne F, Ychou M, Bouche O, Guimbaud R, Becouarn Y, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med. 2011;364:1817–25.
13. Shirasaka T. Development history and concept of an oral anticancer agent S-1 (TS-1): its clinical usefulness and future vistas. Jpn J Clin Oncol. 2009;39:2–15.
14. Ueno H, Ioka T, Ikeda M, Ohkawa S, Yanagimoto H, Boku N, et al. Randomized phase III study of gemcitabine plus S-1, S-1 alone, or gemcitabine alone in patients with locally advanced and metastatic pancreatic cancer in Japan and Taiwan: gEST study. J Clin Oncol. 2013;31:1640–8.
15. Moss DJ, Misko IS, Burrows SR, Burman K, McCarthy R, Sculley TB. Cytotoxic T-cell clones discriminate between A- and B-type Epstein-Barr virus transformants. Nature. 1988;331:719–21.
16. Kimura Y, Tsukada J, Tomoda T, Takahashi H, Imai K, Shimamura K, et al. Clinical and immunologic evaluation of dendritic cell-based immunotherapy in combination with gemcitabine and/or S-1 in patients with advanced pancreatic carcinoma. Pancreas. 2012;41:195–205.
17. Seliger B. Novel insights into the molecular mechanisms of HLA class I abnormalities. Cancer Immunol Immunother. 2012;61:249–54.
18. Plate JM, Harris JE. Immunobiotherapy directed against mutated and aberrantly expressed gene products in pancreas cancer. J Cell Biochem. 2005;94:1069–77.
19. Goydos JS, Elder E, Whiteside TL, Finn OJ, Lotze MT. A phase I trial of a synthetic mucin peptide vaccine. Induction of specific

immune reactivity in patients with adenocarcinoma. J Surg Res. 1996;63:298–304.
20. Karanikas V, Hwang LA, Pearson J, Ong CS, Apostolopoulos V, Vaughan H, et al. Antibody and T cell responses of patients with adenocarcinoma immunized with mannan-MUC1 fusion protein. J Clin Invest. 1997;100:2783–92.
21. Gilewski T, Adluri S, Ragupathi G, Zhang S, Yao TJ, Panageas K, et al. Vaccination of high-risk breast cancer patients with mucin-1 (MUC1) keyhole limpet hemocyanin conjugate plus QS-21. Clin Cancer Res. 2000;6:1693–701.
22. Ramanathan RK, Lee KM, McKolanis J, Hitbold E, Schraut W, Moser AJ, et al. Phase I study of a MUC1 vaccine composed of different doses of MUC1 peptide with SB-AS2 adjuvant in resected and locally advanced pancreatic cancer. Cancer Immunol Immunother. 2005;54:254–64.
23. Yamamoto K, Ueno T, Kawaoka T, Hazama S, Fukui M, Suehiro Y, et al. MUC1 peptide vaccination in patients with advanced pancreas or biliary tract cancer. Anticancer Res. 2005;25:3575–9.
24. Rong Y, Qin X, Jin D, Lou W, Wu L, Wang D, et al. A phase I pilot trial of MUC1-peptide-pulsed dendritic cells in the treatment of advanced pancreatic cancer. Clin Exp Med. 2012;12:173–80.
25. Gjertsen MK, Buanes T, Rosseland AR, Bakka A, Gladhaug I, Soreide O, et al. Intradermal ras peptide vaccination with granulocyte-macrophage colony-stimulating factor as adjuvant: clinical and immunological responses in patients with pancreatic adenocarcinoma. Int J Cancer. 2001;92:441–50.
26. Abou-Alfa GK, Chapman PB, Feilchenfeldt J, Brennan MF, Capanu M, Gansukh B, et al. Targeting mutated K-ras in pancreatic adenocarcinoma using an adjuvant vaccine. Am J Clin Oncol. 2011;34:321–5.
27. Vasef MA, Ross JS, Cohen MB. Telomerase activity in human solid tumors. Diagnostic utility and clinical applications. Am J Clin Pathol. 1999;112:S68–75.
28. Schnabl B, Choi YH, Olsen JC, Hagedorn CH, Brenner DA. Immortal activated human hepatic stellate cells generated by ectopic telomerase expression. Lab Invest. 2002;82:323–33.
29. Bernhardt SL, Gjertsen MK, Trachsel S, Moller M, Eriksen JA, Meo M, et al. Telomerase peptide vaccination of patients with non-resectable pancreatic cancer: a dose escalating phase I/II study. Br J Cancer. 2006;95:1474–82.
30. Miyazawa M, Ohsawa R, Tsunoda T, Hirono S, Kawai M, Tani M, et al. Phase I clinical trial using peptide vaccine for human vascular endothelial growth factor receptor 2 in combination with gemcitabine for patients with advanced pancreatic cancer. Cancer Sci. 2010;101:433–9.
31. Smith JP, Fantaskey AP, Liu G, Zagon IS. Identification of gastrin as a growth peptide in human pancreatic cancer. Am J Physiol. 1995;268:R135–41.
32. Gilliam AD, Watson SA. G17DT: an antigastrin immunogen for the treatment of gastrointestinal malignancy. Expert Opin Biol Ther. 2007;7:397–404.
33. Gilliam AD, Broome P, Topuzov EG, Garin AM, Pulay I, Humphreys J, et al. An international multicenter randomized controlled trial of G17DT in patients with pancreatic cancer. Pancreas. 2012;41:374–9.
34. Srivastava PK. Immunotherapy of human cancer: lessons from mice. Nat Immunol. 2000;1:363–6.
35. Maki RG, Livingston PO, Lewis JJ, Janetzki S, Klimstra D, Desantis D, et al. A phase I pilot study of autologous heat shock protein vaccine HSPPC-96 in patients with resected pancreatic adenocarcinoma. Dig Dis Sci. 2007;52:1964–72.
36. Laheru D, Jaffee EM. Immunotherapy for pancreatic cancer - science driving clinical progress. Nat Rev Cancer. 2005;5:459–67.
37. Hodge JW, Sabzevari H, Yafal AG, Gritz L, Lorenz MG, Schlom J. A triad of costimulatory molecules synergize to amplify T-cell activation. Cancer Res. 1999;59:5800–7.
38. Kaufman HL, Kim-Schulze S, Manson K, DeRaffele G, Mitcham J, Seo KS, et al. Poxvirus-based vaccine therapy for patients with advanced pancreatic cancer. J Transl Med. 2007;5:60.
39. Brockstedt DG, Giedlin MA, Leong ML, Bahjat KS, Gao Y, Luckett W, et al. Listeria-based cancer vaccines that segregate immunogenicity from toxicity. Proc Natl Acad Sci. 2004;101:13832–7.
40. Hassan R, Laszik ZG, Lerner M, Raffeld M, Postier R, Brackett D. Mesothelin is overexpressed in pancreaticobiliary adenocarcinomas but not in normal pancreas and chronic pancreatitis. Am J Clin Pathol. 2005;124:838–45.
41. Le DT, Brockstedt DG, Nir-Paz R, Hampl J, Mathur S, Nemunaitis J, et al. A live-attenuated Listeria vaccine (ANZ-100) and a live-attenuated Listeria vaccine expressing mesothelin (CRS-207) for advanced cancers: phase I studies of safety and immune induction. Clin Cancer Res. 2012;18:858–68.
42. Kobayashi M, Shimodaira S, Nagai K, Ogasawara M, Takahashi H, Abe H, et al. Prognostic factors related to add-on dendritic cell vaccines on patients with inoperable pancreatic cancer receiving chemotherapy: a multicenter analysis. Cancer Immunol Immunother. 2014;63:797–806.
43. Nishida S, Koido S, Takeda Y, Homma S, Komita H, Takahara A, et al. Wilms tumor gene (WT1) peptide-based vaccine combined with gemcitabine for patients with advanced pancreatic cancer. J Immunother. 2014;37:105–14.
44. Taniuchi K, Nakagawa H, Nakamura T, Eguchi H, Ohigashi H, Ishikawa O, et al. Down-regulation of RAB6KIFL/KIF20A, a kinesin involved with membrane trafficking of discs large homologue 5, can attenuate growth of pancreatic cancer cell. Cancer Res. 2005;65:105–12.
45. Asahara S, Takeda K, Yamao K, Maguchi H, Yamaue H. Phase I/II clinical trial using HLA-A24-restricted peptide vaccine derived from KIF20A for patients with advanced pancreatic cancer. J Transl Med. 2013;11:291.
46. Suzuki N, Hazama S, Ueno T, Matsui H, Shindo Y, Iida M, et al. A phase I clinical trial of vaccination with KIF20A-derived peptide in combination with gemcitabine for patients with advanced pancreatic cancer. J Immunother. 2014;37:36–42.
47. Albers GH, Fleuren G, Escribano MJ, Nap M. Immunohistochemistry of CEA in the human pancreas during development, in the adult, chronic pancreatitis, and pancreatic adenocarcinoma. Am J Clin Pathol. 1988;90:17–22.
48. Geynisman DM, Zha Y, Kunnavakkam R, Aklilu M, Catenacci DV, Polite BN, et al. A randomized pilot phase I study of modified carcinoembryonic antigen (CEA) peptide (CAP1-6D)/montanide/GM-CSF-vaccine in patients with pancreatic adenocarcinoma. J Immunother Cancer. 2013;1:8.
49. Tamm I, Wang Y, Sausville E, Scudiero DA, Vigna N, Oltersdorf T, et al. IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. Cancer Res. 1998;58:5315–20.
50. Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med. 1997;3:917–21.
51. Hirohashi Y, Torigoe T, Maeda A, Nabeta Y, Kamiguchi K, Sato T, et al. An HLA-A24-restricted cytotoxic T lymphocyte epitope of a tumor-associated protein, survivin. Clin Cancer Res. 2002;8:1731–9.
52. Kameshima H, Tsuruma T, Kutomi G, Shima H, Iwayama Y, Kimura Y, et al. Immunotherapeutic benefit of alpha-interferon (IFNalpha) in survivin2B-derived peptide vaccination for advanced pancreatic cancer patients. Cancer Sci. 2013;104:124–9.
53. Yutani S, Komatsu N, Yoshitomi M, Matsueda S, Yonemoto K, Mine T, et al. A phase II study of a personalized peptide vaccination for chemotherapy-resistant advanced pancreatic cancer patients. Oncol Rep. 2013;30:1094–100.

54. Yanagimoto H, Shiomi H, Satoi S, Mine T, Toyokawa H, Yamamoto T, et al. A phase II study of personalized peptide vaccination combined with gemcitabine for non-resectable pancreatic cancer patients. Oncol Rep. 2010;24:795–801.
55. Le TD, Wang-Gillam A, Picozzi JJV, Greten FT, Crocenzi ST, Springett MG, et al. A phase 2, randomized trial of GVAX pancreas and CRS-207 immunotherapy versus GVAX alone in patients with metastatic pancreatic adenocarcinoma: updated results. J Clin Oncol. 2014;32(suppl 3):177.
56. Brennan GT, Relias V, Saif MW. Novel agents for the treatment of pancreatic cancer. JOP. 2014;15:110–3.
57. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer. 1981;47:207–14.
58. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst. 2000;92:205–16.
59. Choi JH, Ahn MJ, Rhim HC, Kim JW, Lee GH, Lee YY, et al. Comparison of WHO and RECIST criteria for response in metastatic colorectal carcinoma. Cancer Res Treat. 2005;37:290–3.
60. Hoos A, Parmiani G, Hege K, Sznol M, Loibner H, Eggermont A, et al. A clinical development paradigm for cancer vaccines and related biologics. J Immunother. 2007;30:1–15.
61. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbe C, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res. 2009;15:7412–20.
62. Myc LA, Gamian A, Myc A. Cancer vaccines. Any future? Arch Immunol Ther Exp (Warsz). 2011;59:249–59.

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Phase I Clinical Study of Survivin-Derived Peptide Vaccine for Patients with Advanced Gastrointestinal Cancers

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Abstract

Survivin is a member of the Inhibitor of Apoptosis Protein (IAP) family. It is expressed in fetal tissues but not in normal adult tissues. Since Survivin is over expressed in various types of tumor tissues as well as tumor cell lines, it is considered to be suitable as a target antigen for cancer vaccine therapy. We identified an HLA-A24-restricted antigenic peptide, SVN-2B (AYACNTSTL), derived from a splicing variant of Survivin-2B. In the present study, we carried out a phase I clinical study assessing the safety and efficacy of vaccination with the peptide in patients having advanced gastrointestinal cancer. Vaccinations with 0.1mg, 1.0mg, or 3.0mg doses of the SVN-2B peptide were given subcutaneously four times at 14-day intervals. In 20 patients who received at least one vaccination, grade 1 and grade 2 treatment-related adverse events were observed, including injection site extravasation (grade 2), injection site reaction (grade 1), skin induration (grade 1) and fever (grade 1). No severe adverse event was observed in any patient. Based on tumor size evaluated by computed tomography, eight of the 15 patients who completed the vaccination schedule were considered to have stable disease as assessed by the RECIST criteria. Analysis of peripheral blood lymphocytes using HLA-A24/peptide tetramers revealed the highest increase of SVN-2B-specific cytotoxic T lymphocyte frequency in the 1.0mg dose group. The present clinical study indicates that SVN-2B peptide vaccination is safe and can be considered a potent immunotherapy for HLA-A24-positive gastrointestinal cancer patients.

Keywords

Survivin, Cancer vaccine, Gastrointestinal cancer, Tetramer, Phase I trial

Abbreviations

IAP: Inhibitor of Apoptosis Protein, CTLs: Cytotoxic T lymphocytes, HLA: Human Leukocyte Antigen, CT: Computed Tomography, PBLs: Peripheral Blood Lymphocytes, AEs: Adverse Events, HIV: Human Immunodeficiency Virus, PD: Progressive Disease, SD: Stable Disease, IFN: Interferon

Introduction

Cytotoxic T lymphocytes (CTLs) can recognize MHC class I-bound peptides derived from tumor antigens in cancer cells. Following the first report of the identification of a human tumor antigen, melanoma antigen-1 (MAGE-1), in 1991 [1] a large number of antigenic peptides from various human cancers have been identified [2-7]. They have been employed in immunotherapy for cancer and clinical trials of peptide-based vaccine therapies have taken place [8-11].

We have identified a human leukocyte antigen (HLA)-A24-restricted antigenic peptide, SVN-2B (AYACNTSTL), which was derived from the exon 2B-encoded region of Survivin-2B, a splicing variant of Survivin [12]. Survivin is a member of the inhibitor of apoptosis protein (IAP) family with a single baculovirus IAP repeat domain [13]. It is expressed during fetal development but undetectable in terminally differentiated normal adult tissues. In contrast to normal tissues, Survivin and Survivin-2B are expressed in transformed cell lines and in most common cancers, including gastrointestinal cancer and pancreatic cancer [13,14]. We reported previously that SVN-2B peptide-specific CTLs were increased by

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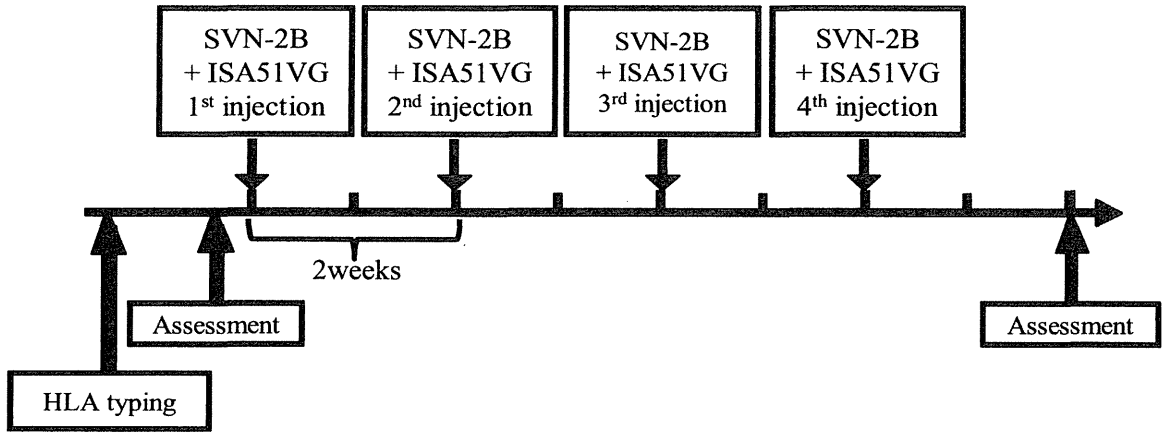

Figure 1: Protocols of the clinical study

The SVN-2B peptide at a dose of 0.1mg/1mL, 1mg/1mL, or 3mg/1mL was emulsified with Montanide ISA51VG at a volume of 0.8mL immediately before vaccination. The patients were then vaccinated subcutaneously (s.c.) four times at 14-day intervals. Tumor size and the immunological response were evaluated before treatment and at two weeks after the 4th vaccination.

stimulating peripheral blood lymphocytes (PBLs) of cancer patients with the peptide *in vitro* [15]. The induced CTLs showed specific cytotoxicity against HLA-A24-positive cancer cells [15-17]. We have carried out clinical trials of SVN-2B vaccination. The SVN-2B peptide was given subcutaneously to patients six times or more at biweekly intervals for colon, breast, oral cavity, and urinary bladder cancer patients [18-24]. There were no severe adverse effects and, clinically, certain patients showed reductions in tumor markers and tumor size as assessed by Computed Tomography (CT). In the present clinical study, we reevaluated the safety and efficacy of SVN-2B vaccination in accordance with good clinical practice guidelines and evaluated the optimal dose of the peptide.

Methods

Patient selection

The study protocol was approved by the Institutional Review Board of Sapporo Medical University. All patients gave informed consent before being enrolled. This study was conducted in accordance with the International Conference on Harmonisation E6 requirements for Good Clinical Practice and with the ethical principles outlined in the Declaration of Helsinki.

Patients enrolled in this study were required to conform to the following criteria: (1) to have histologically confirmed gastrointestinal, bile duct, or pancreatic cancer, (2) to be HLA-A*2402 positive, (3) to have Survivin-positive cancer tissue confirmed by immunohistochemical staining, (4) to be between 20 and 85 years old, (5) to have lesions measureable by CT at the time of registration, (6) to have a history of standard chemotherapy, (7) to have grade 0 or 1 in Eastern Cooperative Oncology Group (ECOG) performance status, and (8) to have no serious organ failure within 30 days at the time of registration.

Exclusion criteria included: (1) prior cancer therapy such as chemotherapy, radiation therapy or other immunotherapy within the previous 4 weeks, (2) presence of other cancers that might influence the prognosis, (3) administration of immunosuppressive drugs such as systemic steroid therapy, (4) severe cardiac insufficiency, acute infection, or hematopoietic failure, (5) uncontrollable diabetes or hypertension, (6) pregnancy or ongoing breast-feeding, and (7) unsuitability for the trial based on clinical judgment. In addition, patients with a high frequency of the peptide-specific CTLs at the time of registration were excluded since such patients were poor responders to the vaccine in our previous studies [23,24]. The number of the HLA-A24/SVN-2B peptide tetramer-positive CTLs per 10,000 CD8-positive T cells (CTLpre) was analyzed at the time of registration and patients who had a value of log10 (1+CTLpre) higher than 1.6 were excluded.

Peptide preparation

The peptide SVN-2B with the sequence **AYACNTSTL** was prepared under good manufacturing practice conditions by PolyPeptide Laboratories San Diego (San Diego, CA, USA). The identity of the peptide was confirmed by mass spectral analysis, and the purity was shown to be more than 98% as assessed by high pressure liquid chromatography analysis. The peptide was supplied as a freeze-dried, sterile white powder. It was dissolved in 1.0 ml of physiological saline (Ohtsuka Pharmaceutical Co., Ltd., Tokyo, Japan) and stored at -80°C until just before use.

Patient treatment

This study was carried out as an open-label, randomized parallel group study at the Department of Surgery, Surgical Oncology and Science of Sapporo Medical University Hospital to evaluate the safety and efficacy of the SVN-2B peptide vaccine for patients who had advanced or recurrent gastrointestinal or pancreatic cancer (UMIN000008611). The patients were randomly assigned into the following three dosage groups: group 1 patients received 0.1mg, group 2 received 1.0mg and group 3 received 3mg. Each group included five patients. SVN-2B at a dose of 0.1mg/1mL, 1mg/1mL, or 3mg/1mL was emulsified with Montanide ISA51VG (Seppic, Paris, France) at a volume of 0.8mL immediately before vaccination. The patients were then vaccinated subcutaneously (s.c.) four times at 14-day intervals (Figure 1).

Toxicity evaluation

Patients were examined closely for signs of toxicity during and after vaccination. Adverse events (AEs) were recorded using CTCAE (version 4.03) criteria and graded for severity.

Clinical response evaluation

Physical and hematological examinations were conducted before and after each vaccination. Changes in tumor marker levels (CEA and CA19-9) were evaluated by comparison of the serum levels before the first vaccination and those after the fourth vaccination. Tumor size was evaluated by CT scans before treatment and at two weeks after the fourth vaccination (Figure 1). The antitumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST: version 1.1) guideline [25]. Briefly, a complete response (CR) was defined as complete disappearance of all measurable and evaluable disease. A partial response (PR) was defined as a >=30%

Table1: Profiles of patients in the full analysis set for safety assessment (N=20)

Clinical variables		0.3 mg (n=7)	1.0 mg (n=7)	3.0 mg (n=6)	Total (n=20)
Gender	Men: Women	2:5	5:2	3:3	10:10
Age	Median (min-max)	69.5 (53-80)	63.0 (51-84)	64.4 (41-66)	65.1(41-84)
Type of cancer	Pancreatic cancer	5	2	5	12
	Colon cancer	2	3	1	6
	Gastric cancer	0	1	0	1
	Bile duct cancer	0	1	0	1
Metastasis	(positive: negative)	7:0	5:2	5:1	17:3
Prior surgery	(positive: negative)	4:3	5:2	3:3	12:8
Prior radiation therapy	(positive: negative)	2:5	2:5	3:3	7:13
Prior chemotherapy	(positive: negative)	6:1	6:1	6:0	18:2
ECOG PS	(0:1)	1:6	1:6	2:4	4:16
Treatment-related AEs					
Fever	Grade 1	1			1
Injection site extravasation	Grade 2			1	1
Injection site reaction	Grade 1	1		1	2
Skin induration	Grade 1	1	1		2

decrease from baseline in the size of all measurable lesions (sum of maximal diameters). Progressive disease (PD) was defined as an increase in the sum of maximal diameters by at least 20% or the appearance of new lesions. Stable disease (SD) was defined as the absence of criteria matching those for CR, PR or PD. Patients who received fewer than four vaccinations were excluded from clinical response evaluations in this study.

In vitro stimulation of PBLs

PBLs were isolated by Ficoll-Conray density gradient centrifugation using Lymphoprep (AXIS-SHIELD, Oslo, Norway). They were then frozen and stored at -80°C. The frozen PBLs were thawed and incubated in the presence of 40μg/mL SVN-2B in AIM-V medium (Life Technologies, Carlsbad, CA, USA) containing 10% human serum at room temperature. Next, interleukin-2 was added at a final concentration of 50 U/mL 1 hour, 2 days and 4 days after addition of the peptide. On day 7 of culture, the PBLs were analyzed by tetramer staining assay and ELISPOT assay.

Tetramer staining

FITC-labeled HLA-A*2402/human immunodeficiency virus (HIV)-derived peptide (RYLRDQQLL) and PE-labeled HLA-A*2402/SVN-2B peptide tetramers were purchased from MBL, Inc. (Nagoya, Japan). For flow cytometric analysis, PBLs, which were stimulated *in vitro* as above, were stained with the FITC-labeled tetramer and PE-labeled tetramer at 37°C for 20 min, followed by staining with a PC5-labeled anti-CD8 monoclonal antibody (Beckton Dickinson Biosciences, San Jose, CA, USA) at 4°C for 30 min. The cells were washed twice with PBS before fixation in 1% formaldehyde. Flow cytometric analysis was performed using FACSCalibur and CellQuest software (Beckton Dickinson Biosciences). The frequency of CTL precursors was calculated as the number of HLA-A24/SVN-2B tetramer-positive cells per 10,000 CD8-positive cells.

ELISPOT assay

ELISPOT plates were coated sterilely overnight with an IFN-γ capture antibody (Beckton Dickinson Biosciences) at 4°C. The plates were then washed once and blocked with AIM-V medium containing 10% human serum for 2 h at room temperature. CD8-positive T cells separated from patients' PBLs ($5x10^3$ cells/well), which were stimulated *in vitro* as above, were then added to each well along with HLA-A24-transfected CIR cells (CIR-A24) ($5x10^4$ cells/well) preincubated with SVN-2B (10ng/mL, 100ng/mL, 10μg/mL) or the HIV peptide (RYLRDQQLL) as a negative control. After incubation in a 5% CO_2 humidified chamber at 37°C for 24 h, the wells were washed vigorously five times with PBS and incubated with a biotinylated anti-human IFN-γ detection antibody (Beckton Dickinson Biosciences) and horseradish peroxidase-conjugated avidin. Spots were visualized and analyzed using KS ELISPOT (Carl Zeiss, Jena, Germany).

Immunohistochemistry

Immunohistochemical study of the HLA class I expression in the patients' primary cancer tissues was done with anti-HLA class I heavy chain monoclonal antibody EMR8-5 according to the standard methods described previously [26].

Statistical analysis

All statistical analyses were done using SAS Version 9.3 and JMP Version 11.0 (SAS Institute, Inc.). For the tetramer assay, statistical analysis was performed using a one-sided t-test. Statistical analysis of ELISPOT assay was performed using the student t-test.

Results

Patient profiles

From August 2012 to May 2013, 38 patients were assessed for eligibility and 21 patients were initially enrolled in this trial (Figure 2). However, one patient was withdrawn before the first vaccination due to deterioration of the systemic condition. Twenty patients who received at least one vaccination were evaluated for safety as a full analysis set (FAS). Five patients discontinued halfway through the protocol due to progression of the disease. None of the interruptions was due to treatment-related AEs. Fifteen patients received the complete regimen including four vaccinations and were evaluated for efficacy of the vaccine (Figure 2). The patient profiles are shown in Table 1. The primary malignant tumors of the 20 patients were 12 pancreatic cancers, 6 colon cancers (including 2 appendix cancers), 1 gastric cancer and 1 bile duct cancer.

Safety

Peptide vaccination was well tolerated in all patients. The treatment-related AEs are listed in Table 1. They included injection site extravasation (grade 2), injection site reaction (grade 1), skin induration (grade 1) and fever (grade 1). No serious toxicity-associated adverse event was observed during or after the vaccination.

Clinical responses

Table 2 summarizes the clinical outcomes of the 15 patients who received the complete regimen. CT evaluation of tumor size showed that 8 patients had SD and 7 patients PD, although none had PR or

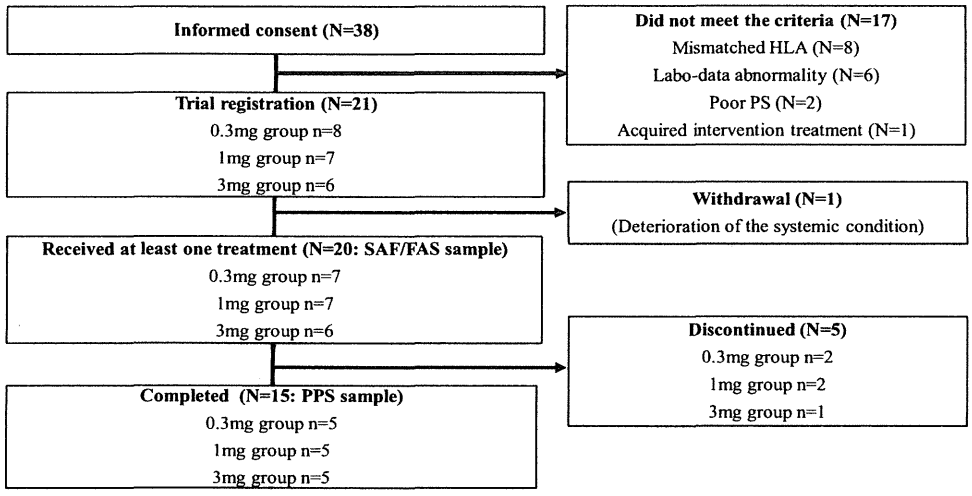

Figure 2: Enrollment of patients

Thirty-eight patients were assessed for eligibility and 21 were initially enrolled in this trial. One patient was withdrawn before the first vaccination due to deterioration of the systemic condition. Twenty patients who received at least one vaccination were evaluated for safety as the full analysis set. Five patients discontinued halfway through the protocol due to progression of the disease. Fifteen patients received the complete regimen and were evaluated for efficacy of the vaccine as the per protocol set. SAF: Safety Analysis Set, FAS: Full Analysis Set, PPS: Per Protocol Set, HLA: Human Leukocyte Antigen, PS: Performance Status.

Table 2: Profiles and clinical outcomes of patients who completed the regimen

Clinical Background						Immunological Response		Antitumor Response		
Dose	Age	Gender	Origin	Status	HLA class I	Tetramer increase	ELISPOT increase	RECIST	CEA	CA19-9
0.3 mg	63	Woman	Pancreas	Inoperable	+	35	-17	**SD**	**Decreased**	**Decreased**
0.3 mg	69	Woman	Pancreas	Inoperable	+	5	-31	**SD**	WNL	Increased
0.3 mg	53	Woman	Pancreas	Post-op	-	7	6	PD	Increased	Increased
0.3 mg	68	Man	Pancreas	Post-op	+	8	17	PD	Increased	Increased
0.3 mg	78	Man	Colon	Post-op	+	-4	2	PD	Increased	Increased
1.0 mg	61	Man	Pancreas	Inoperable	+	21	-1	**SD**	Increased	Increased
1.0 mg	84	Woman	Colon	Post-op	+	28	14	**SD**	Increased	Increased
1.0 mg	69	Man	Stomach	Post-op	+	7	26	**SD**	Increased	Increased
1.0 mg	59	Man	Colon	Post-op	+	29	16	PD	Increased	Increased
1.0 mg	62	Man	Colon	Post-op	+	15	2	PD	Increased	WNL
3.0 mg	41	Woman	Pancreas	Post-op	+	12	158	**SD**	WNL	**Stable**
3.0 mg	66	Man	Pancreas	Inoperable	+	9	19	**SD**	**Decreased**	Increased
3.0 mg	64	Man	Pancreas	Post-op	+	2	-16	**SD**	WNL	**Decreased**
3.0 mg	50	Man	Pancreas	Post-op	+	9	21	PD	WNL	Increased
3.0 mg	64	Woman	Pancreas	Inoperable	+	0	10	PD	Increased	Increased

Post-op: Post-Operative, SD: Stable Disease, PD: Progressive Disease, WNL: Within the Normal Limit

CR. The disease control rate was 53.3%. Among the 8 patients who were defined as having SD, the CEA levels and the CA19-9 levels were decreased or at least stable during vaccination in 2 patients and 3 patients, respectively. The CEA levels stayed within the normal range (0~5.9ng/ml) throughout the study in 4 patients, and the CA19-9 level stayed within the normal range (0~37 U/ml) in one patient. It was noted that all three patients who had undergone immunotherapy before the registration had PD. Moreover, the result for one patient who had HLA class I-negative cancer tissue was also PD.

Tetramer assay and ELISPOT assay

We investigated whether the SVN-2B peptide vaccination could actually induce specific immune responses in the enrolled patients. The peptide-specific CTL frequencies in PBLs before the first vaccination (CTLpre) and after the fourth vaccination (CTLpost) were assessed using the HLA-A24/SVN-2B tetramer, and the tetramer increase (CTLpost-CTLpre) was calculated (Table 2). The HLA-A24/HIV peptide (RYLRDQQLL) tetramer was used as a negative control. SVN-2B-specific CTL frequencies were increased after the vaccination in all patients except two who had undergone immunotherapy before the registration. We compared the tetramer increases between the PD group (non-responders) and SD group (responders). The mean tetramer increase of the SD group was higher than that of the PD group (Figure 3A), although there was no statistical significance