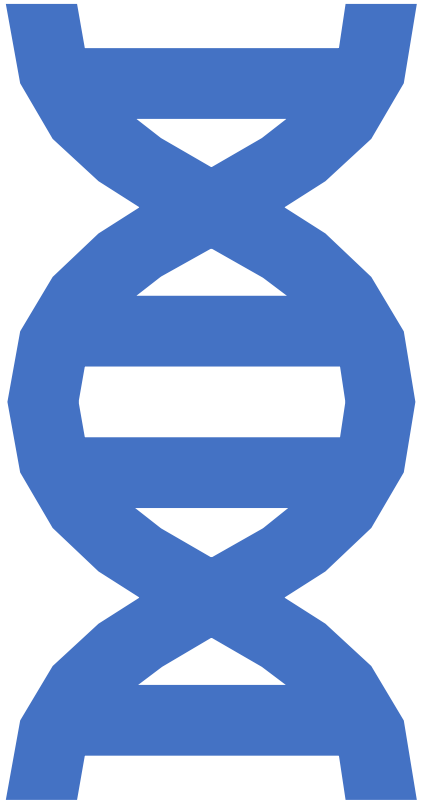


Peptides in Cancer Management

Luis Martinez, MD, MPH

President

Regenera Global

Xanogene Clinic

Objectives

1. Describe the role of peptides in modulating the immune system
2. List peptides which can play a role in cancer management
3. Present mechanisms of action for anti-cancer effects of multiple peptides
4. Discuss protocols and cases

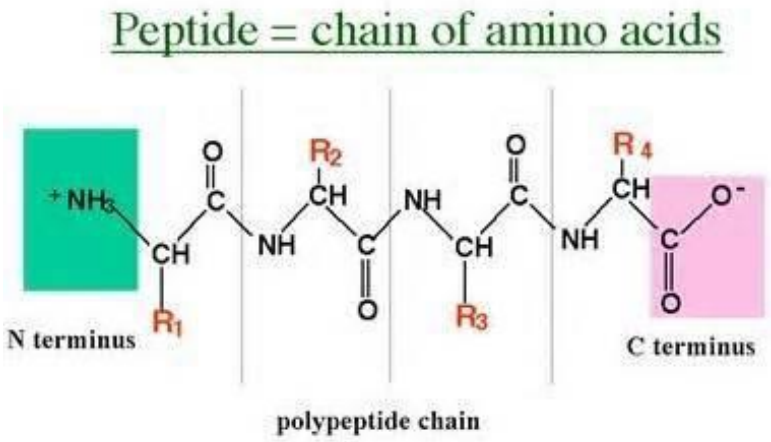
Peptides (basic overview)

Short chains of Amino Acid monomers

Linked by peptide (amide) bonds

Di-, tri-,….poly….. Depending on amount of amino acids joined together

Different from proteins based on size (less tan 70 AAs) and addition of other macromolecules and configuration/bonds (proteins)



Continued

- Derived from multiple sources
- External (milk derived, animal derived, etc)
- Internal (ribosomal, non-ribosomal, etc)

Cancer treatment modalities

- Surgery
- Chemotherapy
- Radiation
- Immunotherapy

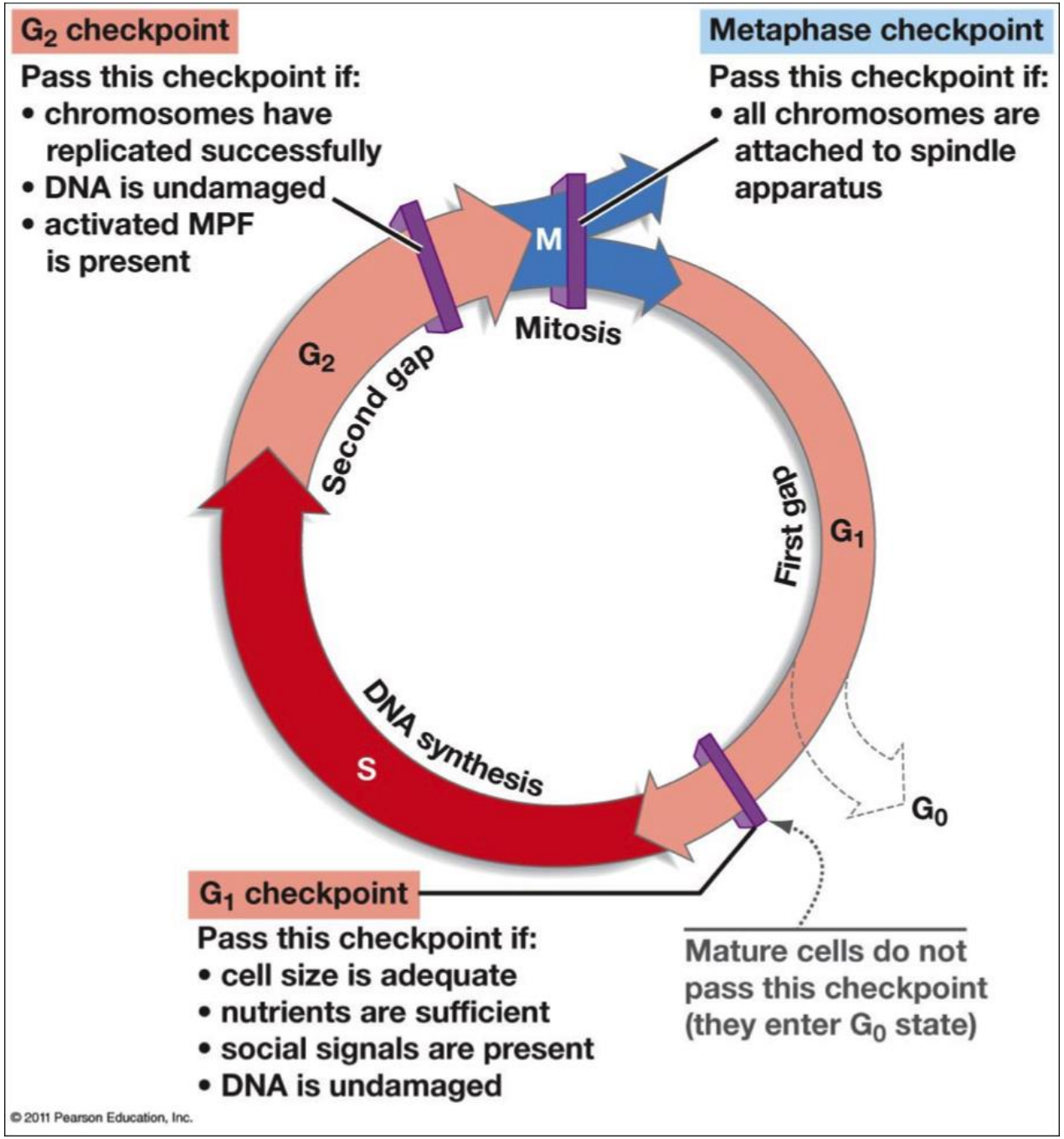
Targeting cancer: Cell growth

Impaired cell growth

Cell cycle checkpoints

- G1-S transition
- G2-M transition
- Exit M phase transition

P53 intervention and apoptosis

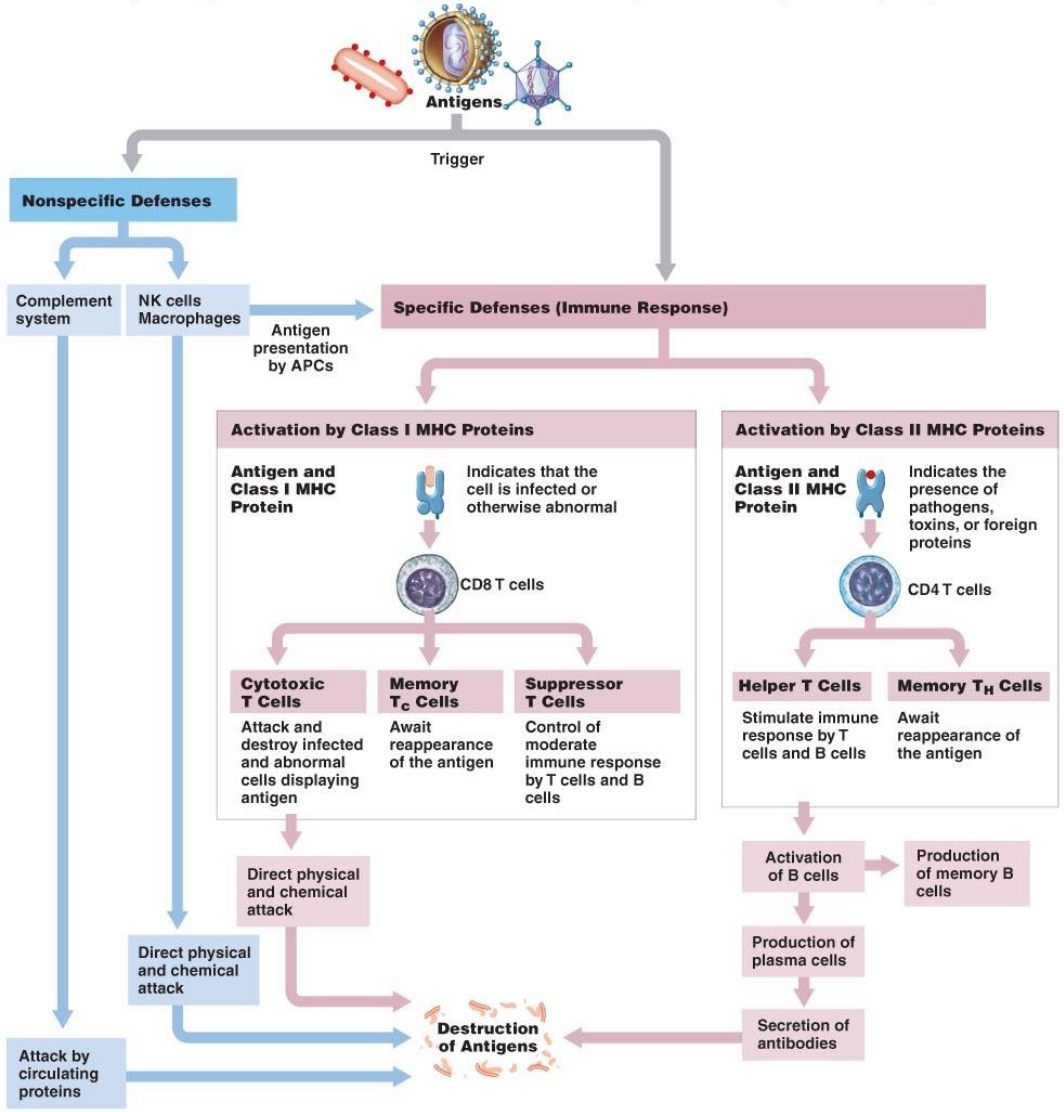


https://classconnection.s3.amazonaws.com/954/flashcards/2108954/png/cell_cycle_checkpoints1353081342979.png

Targeting cancer: Immunotherapy

Serving	Serving as antigens for, stimulating APCs
Modulating	Modulating TH1/TH2
Stimulating	Stimulating NK cells/ Innate immunotherapy
Activating	Activating T cytotoxic cells

The relationships among the elements of the nonspecific defenses and the specific defenses (immune response)



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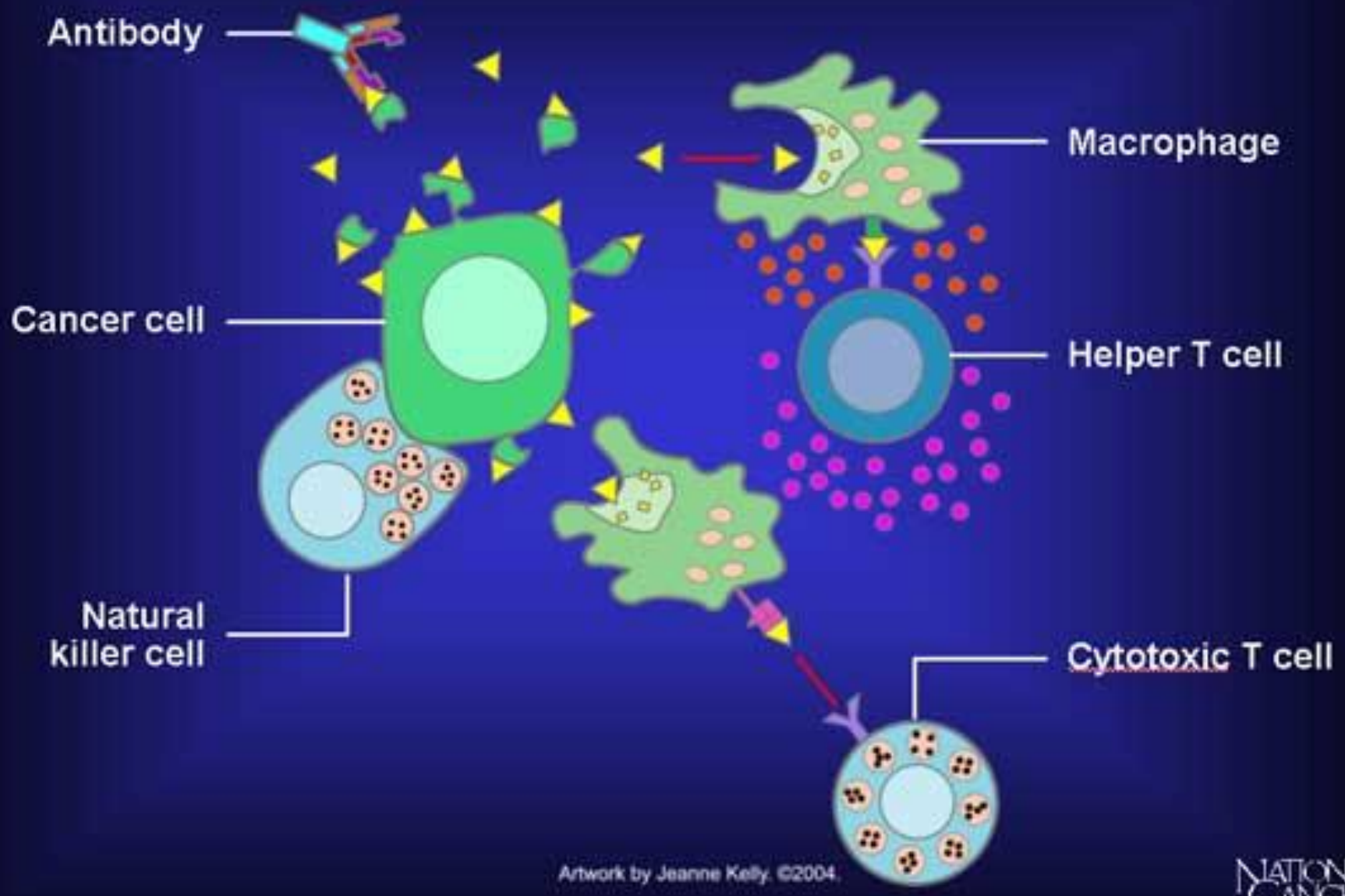
Immunity and Cancer



Artwork by Jeanne Kelly. ©2004.

NATIONAL CANCER INSTITUTE

Targeting Cancer: Direct effects

Cancer cell membrane alterations

Gene expression

P53

Systemic modulation

MTor, AMPK pathways

Peptide applications in Cancer

- Peptide Hormones: (e.g.LHRH agonists/antagonists)
- Peptide Radionuclide carriers
- Peptide vaccines
- Cytotoxic drug carriers
- Direct anticancer peptides

Examples

Table 2: Peptide receptors which have potential in cancer therapy.

Peptide receptors	Receptor subtypes	Expressing tumor type	Targeting agents
Somatostatin	sst1, sst2, sst3, sst4, and sst5	GH-producing pituitary adenoma, paraganglioma, nonfunctioning pituitary adenoma, pheochromocytomas	Radioisotopes, AN-201 (a potent cytotoxic radical 2-pyrrolinodoxorubicin), doxorubicin
Pituitary adenylate cyclase activating peptide (PACAP)	PAC1	Pheochromocytomas and paragangliomas	Radioisotopes, doxorubicin
Vasoactive intestinal peptide (VIP/PACAP)	VPAC1, VPAC2	Cancers of lung stomach, colon, rectum, breast, prostate, pancreatic ducts, liver, and urinary bladder	Radioisotopes, camptothecin
Cholecystokinin (CCK)	CCK1 (formerly CCK-A) and CCK2	Small cell lung cancers, medullary thyroid carcinomas, astrocytomas, and ovarian cancers	Radioisotopes, cisplatin
Bombesin/gastrin-releasing peptide (GRP)	BB1, GRP receptor subtype (BB2), the BB3 and BB4	Renal cell, breast, and prostate carcinomas	Doxorubicin, 2-pyrrolinodoxorubicin
Neurotensin	NTR1, NTR2, NTR3	Small cell lung cancer, neuroblastoma, pancreatic and colonic cancer	Radioisotopes
Substance P	NK1 receptor	Glial tumors	Radioisotopes
Neuropeptide Y	Y1–Y6	Breast carcinomas	Radioisotopes

Peptide based cancer vaccines

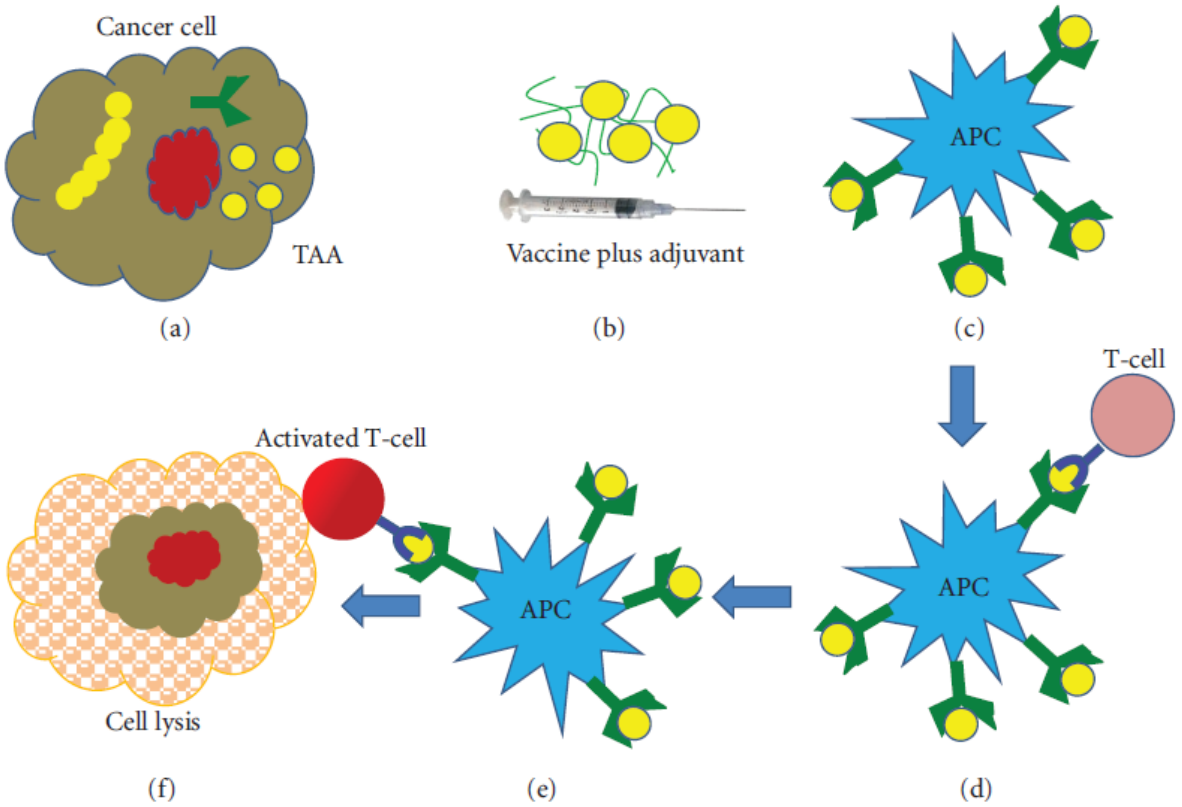


FIGURE 3: Peptide-based cancer vaccines: tumor cells express antigens known as tumor-associated antigens (TAAs) that can be recognized by the host's immune system (a). These TAAs mixed with an adjuvant can be injected into cancer patients in an attempt to induce a systemic immune response (b). The antigen presenting cell (APC) presents the antigen to T cell ((c) and (d)), thereby the T cell is activated (e) which results in the destruction of the cancer cell (f).

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doi:10.1155/2012/967347

PRR THERAPY

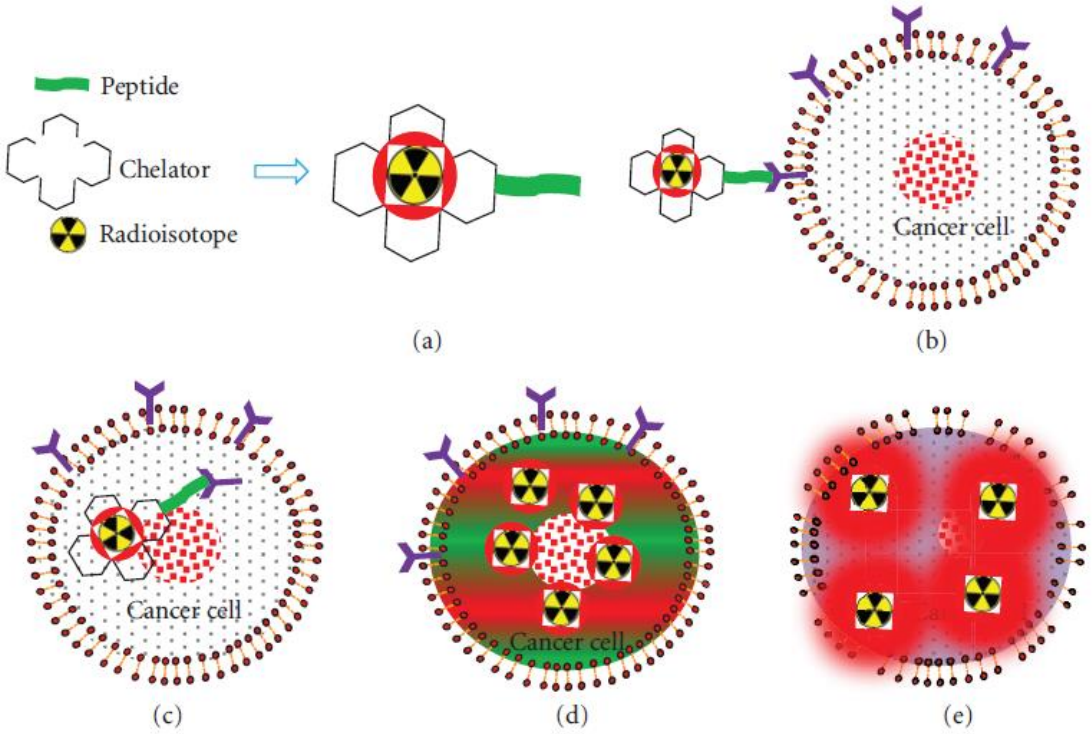


FIGURE 2: Peptide receptor radionuclide therapy (PRRT); radiolabeled somatostatin analogs generally comprise three main parts: a cyclic octapeptide (e.g., Tyr3-octreotide or Tyr3-octreotate), a chelator (e.g., DTPA or DOTA), and a radioactive element. Radioisotopes commonly used in PRRT are 111In, 90Y, and 177Lu.

Direct anti cancer peptides

Stimulate/alter immune response (Immune modulation)

May be involved in passive/active cancer immunotherapy

May also target specific cell growth and apoptosis

Perceived challenges for direct peptide therapy

- Rapidly eliminated
- Recurrent injectable treatments
- Temperature liable
- Costs
- Studies and regulatory hurdles

Anticancer peptides

Endogenous opioid peptides

01

Promote healing

02

Inhibit cell growth

03

Reduce inflammation

Opioid Growth Factor (OGF)

- Opioid Growth Factor (OGF) known as Metkephalin (Met5)
- Endogenous pentapepide
- OGF activates a specific receptor called Opioid Growth Factor receptor (OGFr or ζ-opioid receptor).
- OGF and OGFr axis regulates cell growth in normal and abnormal cells

OGF and cell growth

OGF-OGFr pathway can control the cell cycle

OGF-OGFr upregulation at the translational level produces oncostatic/oncosuppressive effects

OGF-OGFr axis regulates cell proliferation by altering the G1/S phase of the cell cycle through the p16 and p21 cyclin – dependent inhibitory kinases

Metenkephalin production (OGF) stimulates P16 and P21 inhibitory pathways of cancer cell division

OGF Cancer studies

Zagon et al. have studied OGF extensively

Found to kill colon1, pancreas2, squamous cell3, neuroblastoma4, renal cell5, triple neg. breast6, ovarian cancers7 (*in vitro*)

Phase 1 human trial of i.v. OGF in 16 pancreatic cancer patients showed improved survival over standard 5-FU or gemcitabine chemo, with good pain control8

Cancer Lett. 2014 Mar 28;344(2):212-22. doi: 10.1016/j.canlet.2013.10.029. Epub 2013 Nov 26.

Immunotherapy of cancer via mediation of cytotoxic T lymphocytes by methionine enkephalin (MENK).

Li W[1], Chen W[2], Herberman RB[3], Plotnikoff NP[3], Youkilis G[3], Griffin N[3], Wang E[4], Lu C[1], Shan F[5].

Abstract

The aim of this study was to investigate the immunological mechanisms by which synthetic methionine enkephalin (MENK) exerts therapeutic effects on tumor growth. **Our findings in vivo or in vitro show that MENK treatment either in vivo or in vitro could up-regulate the percentages of CD8+T cells, induce markers of activated T cells, increased cytotoxic activity against mouse S180 tumor cells and increase secretion of IFNγ.** In addition, the adoptively transferred CD8+T cells, after either in vitro or in vivo treatment with MENK, result in significantly increased survival of S180 tumor-bearing mice and significant shrinkage in tumor growth. Opioid receptors are detected on normal CD8+T cells and exposure to MENK leads to increased expression of opioid receptors. Interaction between MENK and the opioid receptors on CD8+T cells appears to be essential for the activation of CTL, since the addition of naltrexone (NTX), an opioid receptor antagonist, significantly inhibits all of the effects of MENK. The evidence obtained indicates that the MENK-induced T cell signaling is associated with a significant up-regulation of Ca2+ influx into the cytoplasm and the translocation of NFAT2 into nucleus, and these signaling effects are also inhibited by naltrexone.

Oncol Rep. 2017 Oct;38(4):2132-2140. doi: 10.3892/or.2017.5918. Epub 2017 Aug 24.

Killing effect of methionine enkephalin on melanoma in vivo and in vitro.

Wang DM1, Jiao X2, Plotnikoff NP3, Griffin N3, Qi RQ4, Gao XH4, Shan FP1.
Author information

Abstract

Melanoma is a common cutaneous malignancy, that is also found in specific mucosal sites, and is associated with a poor prognosis. The aim of the present study was to investigate the cytotoxicity of methionine enkephalin (MENK) for B16 melanoma cells in vivo and in vitro. The results of the present study allowed our conclusion that MENK regulates the proliferation of B16 cells, causing cell cycle arrest in the G0/G1 phase and a decrease in the percentage of cells in the S and G2/M phases. Reverse transcription-quantitative polymerase chain reaction demonstrated that MENK increased opioid receptor expression in the B16 cells. **Furthermore, the tumor volume and weight in the MENK-treated group were lower than those in the control group (NS) and MENK and naltrexone (NTX)-treated groups**. **MENK exerted both significant antitumor activity on the growth of B16 cells and a longer survival time in mice**. The mice treated with MENK exhibited an increased ratio of CD4+ to CD8+ T cells as tested by flow cytometry (FCM), resulting in a ratio of 2.03 in the control group, 3.69 in the MENK-treated group, and 2.65 in the MENK and NTX group. Furthermore, a significant increase in plasma levels of IL-2, IFN-γ and TNF-α was revealed as assessed by ELISA. In conclusion, the results of the present study indicate that MENK has a cytotoxic effect on B16 melanoma cells in vitro and in vivo, and suggest a potential mechanism for these bioactivities. Therefore, we posit that MENK should be investigated, not only as a primary therapy for melanoma, but also as an adjuvant therapy in combination with chemotherapies.

Int Immunopharmacol. 2017 Mar;44:61-71. doi: 10.1016/j.intimp.2017.01.004. Epub 2017 Jan 12.

Methionine enkephalin (MENK) mounts antitumor effect via regulating dendritic cells (DCs).

Meng Y[1], Gao X[2], Chen W[3], Plotnikoff NP[4], Griffin N[4], Zhang G[5], Shan F[6].

Abstract

MENK, an endogenous opioid peptide has been reported to have many immunological and antitumor activities. So far the detailed mechanisms of antitumor through regulating DCs by MENK have not been elucidated yet. The aim of this work was to investigate the antitumor mechanisms of MENK via regulating DC. The monitoring methods, such as ELISA, MTS assay, CFSE, Real-time PCR and Western blot were included in our research. We found bone marrow derived dendritic cells (BMDCs) in 36 female C57BL/6 mice treated with MENK enhanced expression of key surface molecules, increased production of critical cytokines reduced endocytosis of FITC-dextran, upregulated TLR4 through MyD88/NF-κB signaling pathway and mounted higher antitumor activity. These observations were further supported by an enhancement of nuclear translocation of the p65NF-κB subunit involved in this process. Surprisingly, mu-opioid receptors were the main participants of this kind of activation, not delta-opioid receptors nor kappa-opioid receptors, and these interactions could be partly blocked by Naltrexone (a kind of opioid antagonist). In vivo study the activated $CD4^+$, $CD8^+$T cells and decreased ability to induce differentiation of $Foxp3^+$ regulatory T cells were detected post treatment of MENK. **Thus, it is concluded that MENK could exert antitumor effect through precisely regulating opioid receptor mediated functions of DCs. In addition, MENK treated DCs may serve as a new immunotherapy approach against tumor.**

Hum Vaccin Immunother. 2014;10(7):1836-40. doi: 10.4161/hv.28804.

Methionine enkephalin (MENK) improves lymphocyte subpopulations in human peripheral blood of 50 cancer patients by inhibiting regulatory T cells (Tregs).

Wang Q[1], Gao X, Yuan Z, Wang Z, Meng Y, Cao Y, Plotnikoff NP, Griffin N, Shan F.

Abstract

MENK, a penta-peptide is considered as being involved in the regulatory feedback loop between the immune and neuroendocrine systems, with marked modulation of various functions of human immune cells. The aim of the present work was to investigate change of lymphocyte subpopulations in peripheral blood of 50 cancer patients before and after treatment with MENK. Peripheral blood mononuclear cells (PBMCs) of peripheral blood from 50 cancer patients were isolated by density gradient centrifugation using Ficoll-Paque solution and cultured with MENK. We measured proliferation of total nucleated cells, subpopulations of individual CD4+T cells, CD8+T cells, CD4+CD25+ regulatory T cells (Treg), natural killer cells (NK) before and after treatment with 10(-12)M MENK in cell culture by flow cytometry (FCM). **Our results indicated that MENK showed a strong inhibiting effect on Treg cells while it stimulated marked proliferation of other lymphocyte subpopulations. All data obtained were of significance statistically. It was therefore concluded that MENK could work as a strong immune booster with great potential in restoring damaged human immune system and we could consider MENK as a drug to treat cancer patients**, whose immune systems are damaged by chemotherapy or radiotherapy. Furthermore we could consider MENK as a chemotherapy additive, which would sustain immune system of cancer patients during the process of chemotherapy to get maximized efficacy with minimized side

Open Access J Clin Trials. 2010 Mar 1;2010(2):37-48.

Opioid growth factor improves clinical benefit and survival in patients with advanced pancreatic cancer.

Smith JP[1], Bingaman SI, Mauger DT, Harvey HH, Demers LM, Zagon IS.

Abstract

BACKGROUND:

Advanced pancreatic cancer carries the poorest prognosis of all gastrointestinal malignancies. Once the tumor has spread beyond the margins of the pancreas, chemotherapy is the major treatment modality offered to patients; however, chemotherapy does not significantly improve survival.

OBJECTIVE:

Opioid growth factor (OGF; [Met(5)]-enkephalin) is a natural peptide that has been shown to inhibit growth of pancreatic cancer in cell culture and in nude mice. The purpose of this study was to evaluate the effects of OGF biotherapy on subjects with advanced pancreatic cancer who failed chemotherapy.

METHODS:

In a prospective phase II open-labeled clinical trial, 24 subjects who failed standard chemotherapy for advanced pancreatic cancer were treated weekly with OGF 250 µg/kg intravenously. Outcomes measured included clinical benefit, tumor response by radiographic imaging, quality of life, and survival.

RESULTS:

Clinical benefit response was experienced by 53% of OGF-treated patients compared to historical controls of 23.8% and 4.8% for gemcitabine and 5-fluorouracil (5-FU), respectively. **Of the subjects surviving more than eight weeks, 62% showed either a decrease or stabilization in tumor size by computed tomography. The median survival time for OGF-treated patients was three times that of untreated patients (65.5 versus 21 days, $p < 0.001$).** No adverse effects on hematologic or chemistry parameters were noted, and quality of life surveys suggested improvement with OGF.

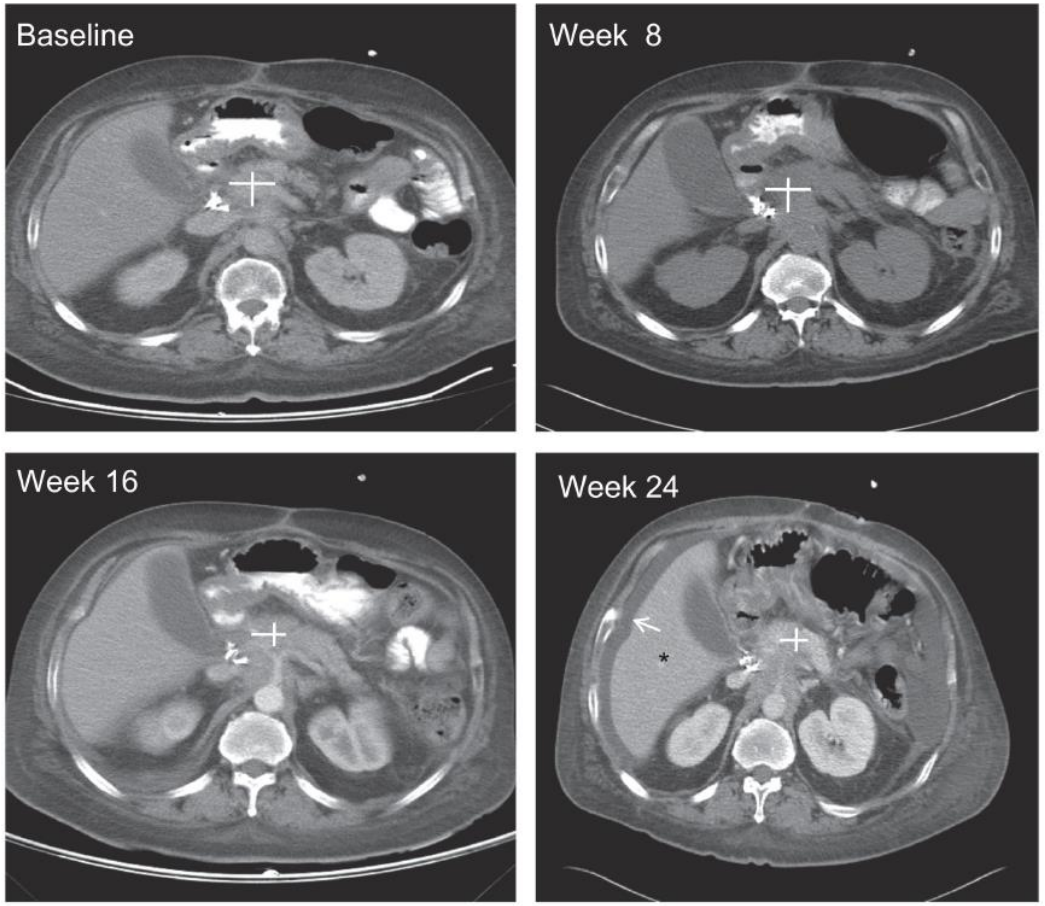


Figure 2.
Opioid growth factor (OGF) decreases and/or stabilizes growth of pancreatic cancer. Radiographic images by computerized tomography are shown demonstrating the sections through the primary pancreatic tumor in the head of the pancreas. The pancreatic tumor (marked with a white +) is shown at baseline and every eight weeks during OGF therapy. The primary tumor size decreased during the study, but at week 24 the patient was noted to have ascites in the peritoneal cavity (arrow) and an 11 mm nodule in the liver (*) suggestive of progression of disease.

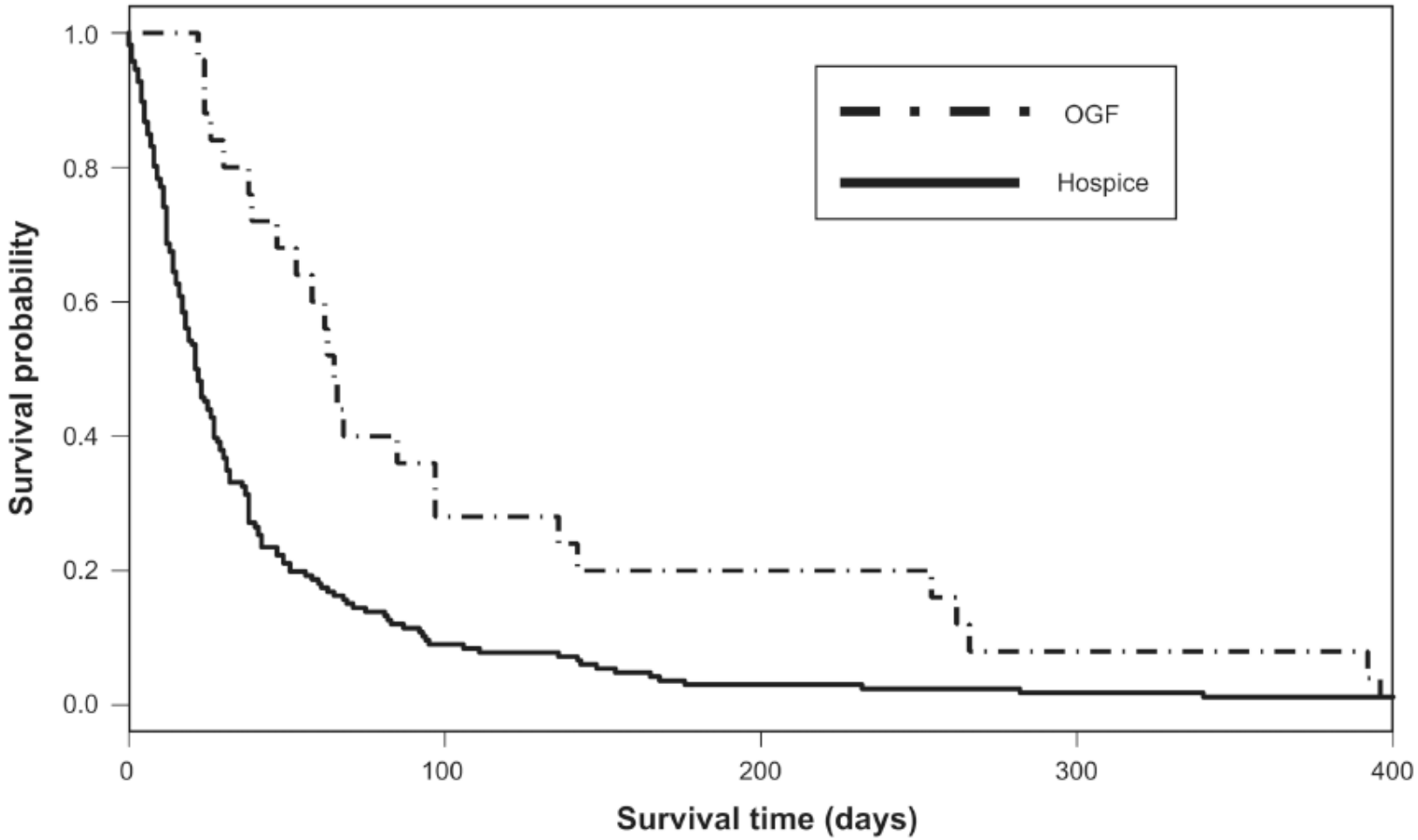


Figure 3.

Survival of advanced pancreatic cancer is improved compared to untreated controls. **A)** Opioid growth factor (OGF)-treated patients survived for 209% longer than untreated controls. **B)** Survival of OGF-treated subjects over time compared to control subjects using the Kaplan–Meier curve. The OGF-treated group differed significantly from controls at $p < 0.001$.

Notes: Data represent means ± standard error of mean. ***Significantly different from hospice controls, $p < 0.001$.

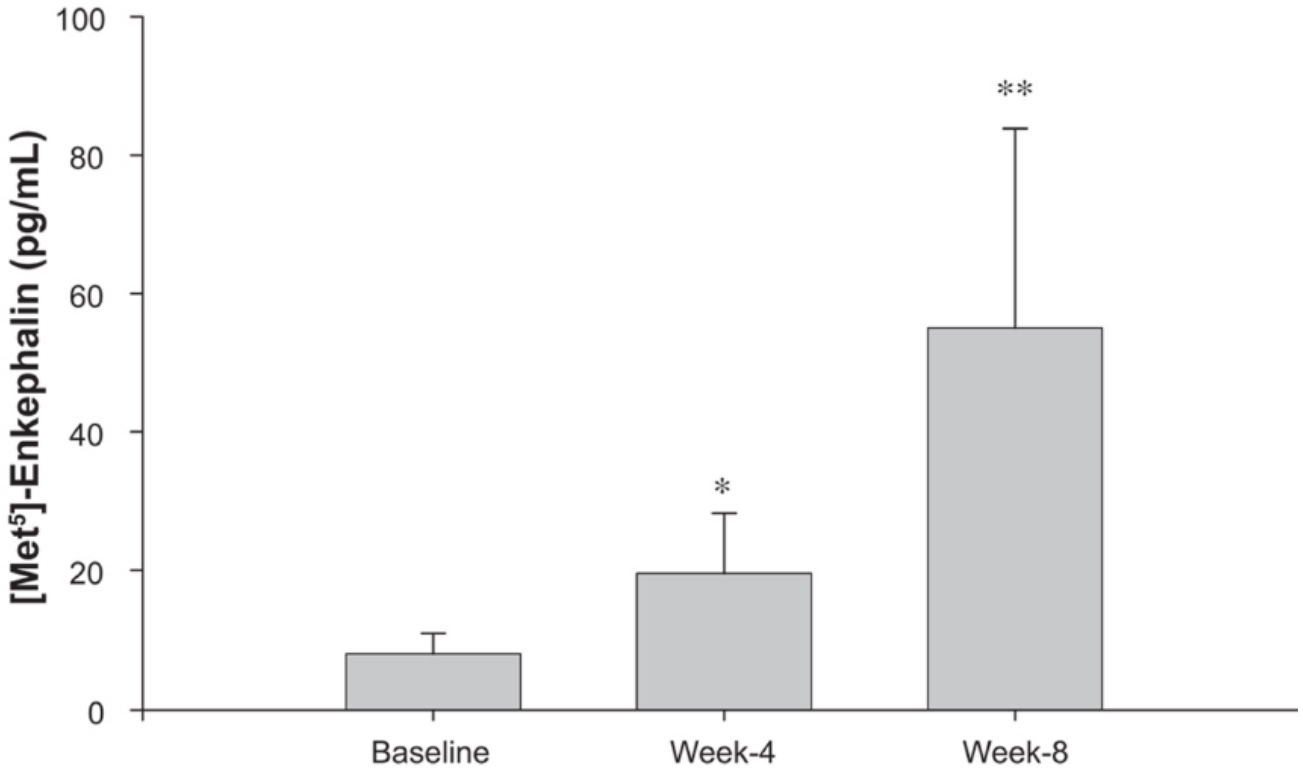


Figure 4.

Plasma [Met^5]-enkephalin levels in opioid growth factor (OGF)-treated patients over time. The antibody used for this assay was highly specific for [Met^5]-enkephalin with little or no cross reactivity with B-endorphin, dynorphin A, ACTH and endothelin-1. The assay range was 10–1280 pg/mL. Between run precision at concentrations of 21 and 636 pg/mL averaged 16% and 11% CV, respectively, and the lower limit of quantitation was 8 pg/mL.

Notes: Data are presented as means ± standard error of mean for baseline, week 4, and week 8 for subjects treated with OGF. Significantly different from baseline are represented by $*p < 0.01$, and $**p < 0.001$.

Dosing

Based on clinical trials as well as direct patient experience

Given s.c. or i.v., 5-15mg per administration

(Calculate at 250mcg/kg; dosing has been studied from weekly to daily administration

Max dose 10mg s.c. b.i.d.

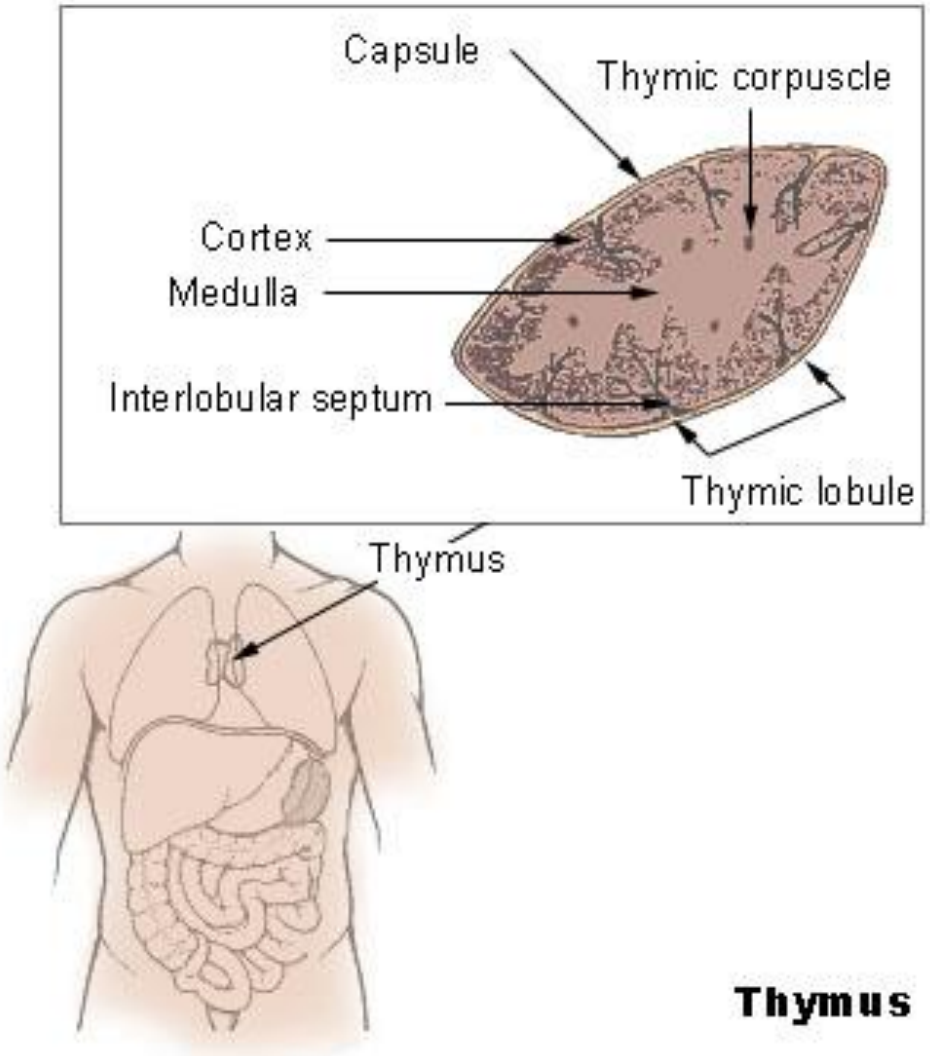
Must be refrigerated

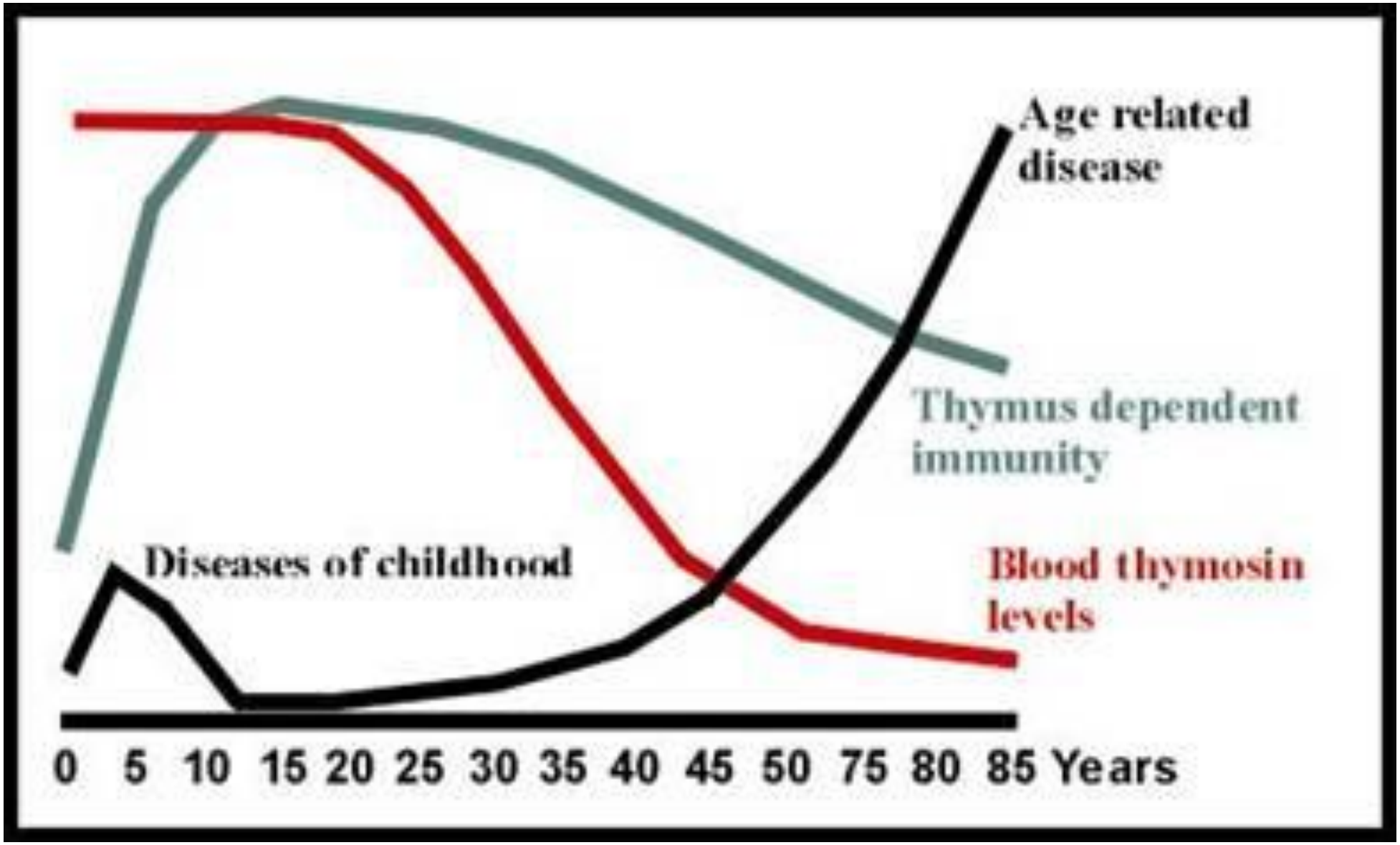
Thymosin Alpha 1 (TA-1)

- Endogenous human peptide
- Derived from prothymosin
- 28 aa fragment
- Helps restore immune function (remember thymic involution and aging)
- Enhances cell mediated immunity
- Increases efficiency of antigen presenting cells

The thymus gland

- Specialized primary lymphoid organ of immune system
- T cell maturation
- Adaptive immune system
- Involutes with aging





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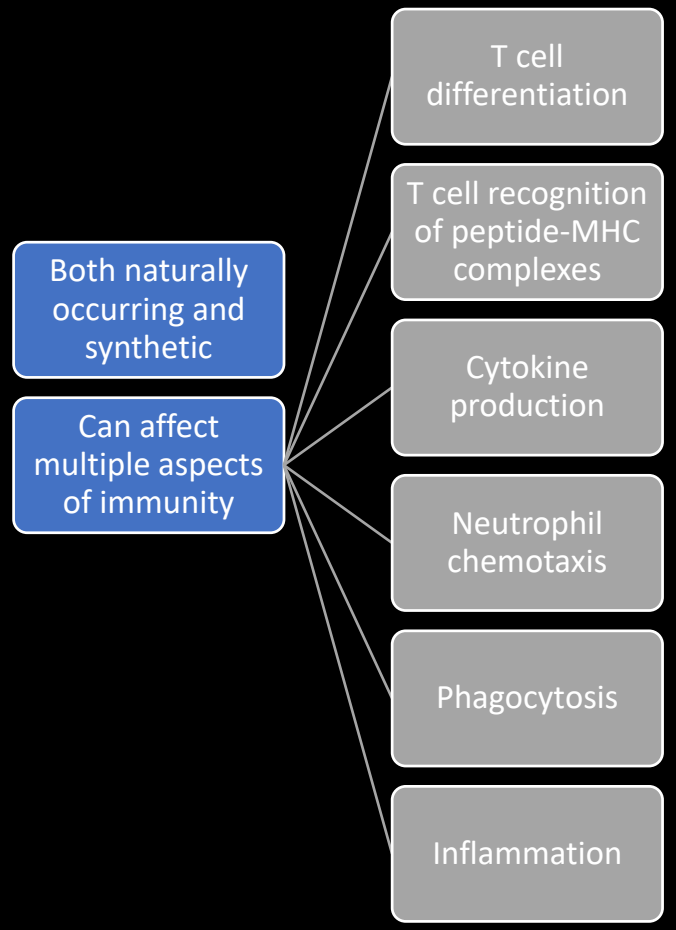
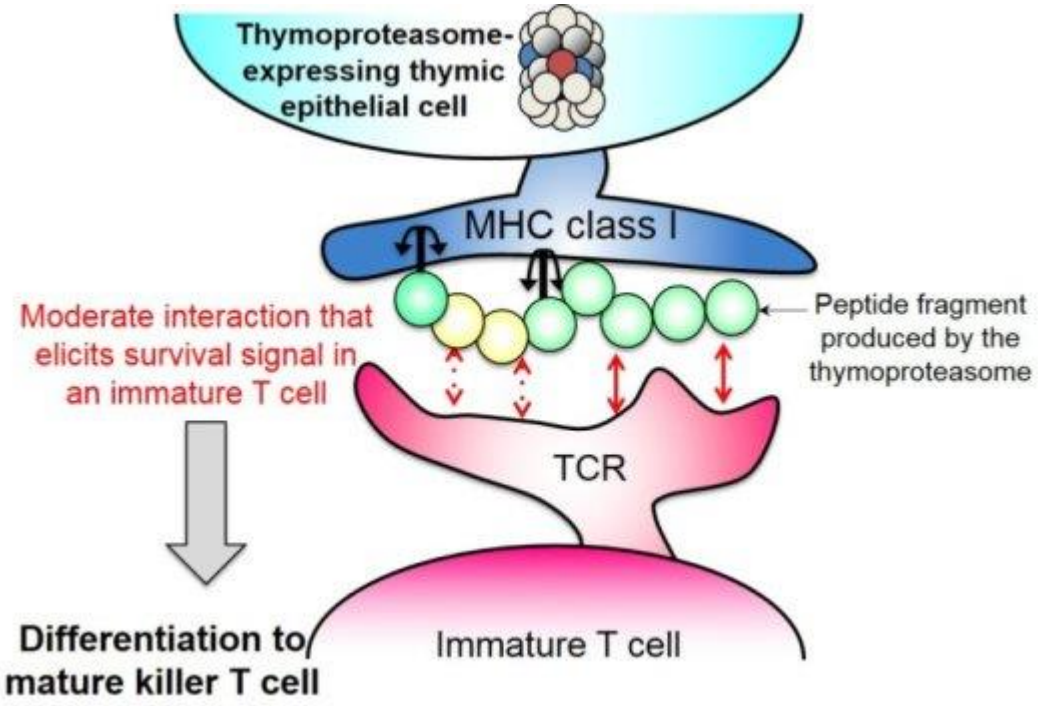
Thymic peptides





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Thymic peptides

Type	Name	Ingredients	Provider	Applied in study
Purified thymus extracts	Thymosin fraction 5	Peptide mixture, range 1-15 kDa	Hoffmann-La Roche	Bedikian 1984; Cohen 1979; Scher 1988; Wara 1981
	Thymostimulin	Peptide mixture, range 1-12 kDa	Serono S.A.	Airoldi 1987; Canovas 1988; Canovas 1991; De Serdio 1997; Del Giacco 1988; Federico 1995; Gonnelli 1995; Guzman 1988; Iaffaioli 1994; Luzi 1984; Macchiarini 1989; Mantovani 1988; Mustacchi 1994; Pavesi 1993; Salvati 1984; Sanchiz 1996
Synthetic thymic peptides	Thymosin α_1	Polypeptide (28 amino acids)	SciClone Pharmaceuticals	Cheng 2004; Gish 2009; Maio 2010; Schulof 1985
	Thymopentin	Oligopeptide (5 amino acids)	Italfarmaco	Gebbia 1994; GISOT 1987

Thymosin Alpha 1 studies

Zhejiang Da Xue Xue Bao Yi Xue Ban. 2003 Aug;32(4):339-41.

[Effect of thymosin alpha 1 on cellular immune function in elderly patients with malignant tumor].

[Article in Chinese]

Yang YM[1], Lu XY, Huang WD, Shen MY.

Author information

Abstract

OBJECTIVE:

To investigate the effect of thymosin alpha 1 on cellular immune function in the elderly patients with malignant tumor.

METHODS:

Thirty patients with malignant tumor were injected with thymosin alpha 1 subcutaneously at the dose of 1.6 mg q.d. for the first month and q.o.d. for the following month. The number of T cell subgroups and the activity of NK cell in peripheral blood were detected and the quality of life of the patients were evaluated before treatment and at the end of treatment.

RESULT:

Treatment of thymosin alpha 1 increased the number of CD4 cells and improved the NK activity, and also improved the quality of life of the elderly patients with malignant tumor. There were no side effects found.

CONCLUSION:

Thymosin alpha 1 can enhance the cellular immune function of the elderly patients with malignant tumor.

Int Immunopharmacol. 2003 Aug;3(8):1145-50.

Thymosin alpha(1) in combination with cytokines and chemotherapy for the treatment of cancer.

Garaci E[1], Pica F, Sinibaldi-Vallebona P, Pierimarchi P, Mastino A, Matteucci C, Rasi G.

Author information

Abstract

Multiple therapeutic approaches have been tested in different experimental tumour models and in human cancers. Most part of them are based on the hypothesis that the inhibition of tumour growth requires a strong immune response in which a main role is played by CTLs. It is known, however, that an efficient CTL response requires expression of tumour antigens, MHC class I surface molecules presentation, expression of different co-stimulatory molecules and a sustained generation and proliferation of specific cytotoxic CD8+ cells with an efficient CD4+ cooperation. In this context, our group has extensively explored a protocol of combined therapy consisting of the use of chemotherapeutic agents associated with thymosin alpha 1 (Talpha 1) and different cytokines, whose efficacy has been demonstrated in experimental models as well as in human cancers. In this manuscript, the main data supporting a pivotal role of Talpha 1 in such combination protocols are reviewed. In particular, a special mention of the molecular mechanisms underlying the effects of Talpha 1 on immune effector cells as well as on target tumour cells is provided. **These data contribute to explain the mechanism of action of Talpha 1, when used in combination therapy, for the treatment of cancer and provide new insights in predicting further possible applications of this peptide in other pathological conditions.**

Large Randomized Study of Thymosin α 1, Interferon Alfa, or Both in Combination With Dacarbazine in Patients With Metastatic Melanoma

Michele Maio, Andrzej Mackiewicz, Alessandro Testori, Uwe Trefzer, Virginia Ferraresi, Jacek Jassem, Claus Garbe, Thierry Lesimple, Bernard Guillot, Pere Gascon, Katalin Gilde, Roberto Camerini, and Francesco Cognetti

A B S T R A C T

Purpose

Thymosin α 1 (Tα1) is an immunomodulatory polypeptide that enhances effector T-cell responses. In this large randomized study, we evaluated the efficacy and safety of combining Tα1 with dacarbazine (DTIC) and interferon alfa (IFN-α) in patients with metastatic melanoma.

Patients and Methods

Four hundred eighty-eight patients were randomly assigned to five treatment groups: DTIC+IFN-α+Tα1 (1.6 mg); DTIC+IFN-α+Tα1 (3.2 mg); DTIC+IFN-α+Tα1 (6.4 mg); DTIC+Tα1 (3.2 mg); DTIC+IFN-α (control group). The primary end point was best overall response at study end (12 months). Secondary end points included duration of response, overall survival (OS), and progression-free survival (PFS). Patients were observed for up to 24 months.

Results

Ten and 12 tumor responses were observed in the DTIC+IFN-α+Tα1 (3.2 mg) and DTIC+Tα1 (3.2 mg) groups, respectively, versus four in the control group, which was sufficient to reject the null hypothesis that $P_0 \leq .05$ (expected response rate of standard therapy) in these two arms. Duration of response ranged from 1.9 to 23.2 months in patients given Tα1 and from 4.4 to 8.4 months in the control group. Median OS was 9.4 months in patients given Tα1 versus 6.6 months in the control group (hazard ratio = 0.80; 95% CI, 0.63 to 1.02; $P = .08$). An increase in PFS was observed in patients given Tα1 versus the control group (hazard ratio = 0.80; 95% CI, 0.63 to 1.01; $P = .06$). Addition of Tα1 to DTIC and IFN-α did not lead to any additional toxicity.

Conclusion

These results suggest Tα1 has activity in patients with metastatic melanoma and provide rationale for further clinical evaluation of this agent.

Cancer Invest. 1994;12(5):491-6.

Thymosin alpha-1 as adjunct for conventional therapy of malignant tumors: a review.

Bepler G[1].

Author information

- [1]Department of Medicine, Duke University Medical Center, Durham, North Carolina.

Abstract

T alpha 1, a 28-amino-acid peptide, is derived from PT alpha, which is an intracellular, nonsecretory protein of unknown function. Both T alpha 1 and PT alpha are found in the blood of normal individuals. Subcutaneous and intramuscular injections of T alpha 1 in doses up to 9.6 mg/m2 are tolerated without side effects, and 0.9 mg/m2 injections raise the serum level approximately 30-fold after 1 hr of administration, which slowly returns to baseline within 24 hr. In vitro, and perhaps in vivo, T alpha 1 restores normal T-cell function. It increases IL-2 production and IL-2 receptors in normal mitogen-stimulated T cells and stimulates IL-3 production in immunocompromised mice. The dose-response relationship for these effects is not linear and may be bimodal. T alpha 1 binds to VIP receptors and inhibits in vitro and xenograft growth of non-SCLC cell lines. In patients with nonbulky carcinomas who have received standard therapy, T alpha 1 is possibly effective in prolonging the time to relapse and in improving survival. At present there is a great need to clearly define the clinical role of T alpha 1 in cancer patients. A major problem encountered in studies with T alpha 1 will, however, be the present lack of knowledge with regard to its mechanism in effecting tumor growth. It is not at all clear whether its immunomodulatory functions, its interaction with VIP receptors, or none of these mechanisms are related to its antineoplastic activities. In addition, the apparent nonlinear dose-response relationship will make it difficult to choose a reasonable dosing schedule for clinical trials. This is particularly apparent in light of the experimental animal data summarized above where a tumor response was seen at doses of 4 micrograms/kg and 400 micrograms/kg but not at 0.4 microgram/kg and 40 micrograms/kg. This dose range could conceivably be given to humans since 9.6 mg/m2, the maximum dose given to humans without major side effects to date, is roughly equivalent to 250 micrograms/kg. At this time a reasonable clinical approach would be a well-designed risk factor stratified phase III clinical trial using 0.9 mg/m2 T alpha 1 subcutaneously twice a week compared to a control group to substantiate the data reported by Schulof et al. Before such data are available, T alpha 1 should not be used in clinical oncology.

Effect of thymosin α_1 on the phenotypic and functional maturation of dendritic cells from children with acute lymphoblastic leukemia

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Abstract. To determine the effect of thymosin α_1 (Tα1) on the phenotypic and functional maturation of HL-60 cells, freeze-thaw antigen-loaded dendritic cells (DCs) were derived from peripheral blood mononuclear cells (PBMCs) of children with acute lymphoblastic leukemia (ALL). The DCs were generated from the PBMC samples that were collected from the PB of 10 consecutive ALL children. On day 3 of culturing, the cells in the antigen + no Tα1 (AN) and antigen + Tα1 (AT) groups were incubated with 100 μl lysates obtained from freeze-thaw cycling. After 5 days of incubation, the AT group was administered with 100 ng/ml Tα1. On day 8, the DCs were stained with fluorescein isothiocyanate-conjugated cluster of differentiation (CD)1a, CD83 and HLA-DR antibodies and analyzed by flow cytometry. In addition, the killing activity of cytotoxic T lymphocytes (CTLs) from the different groups on wild-type leukemia cells was measured. The DCs in the AT group exhibited more apparent, characteristic dendritic morphologies than the control and AN group DCs. Furthermore, the lowest expression level of CD1a, and the highest expression of CD83 and HLA-DR were observed in the AT group when compared with the AN and control groups ($P<0.05$). The lactate dehydrogenase release assay demonstrated that the killing rate of CTL in the AT group was significantly higher than that in the control and AN groups ($P<0.01$). Thus, Tα1 may markedly promote the phenotypic and functional maturation of DCs, and may serve as a suitable immunomodulator of DC-based immunotherapy for treatment of hematological malignancies.

Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood malignant disease, and accounts for the greatest percent of malignancies in children newly diagnosed with cancer in the USA (1,2). Following chemotherapy and hematopoietic stem cell transplantation, novel therapeutic strategies have been developed to improve the complete remission (CR) rate and overall survival of ALL patients (3,4). However, significant toxicity, relapse due to a state of minimal residual disease (MRD) and transplant-associated mortality limit the efficacy of allogeneic stem cell transplantation (5). Therefore, the development of additional immunotherapeutic strategies that selectively recognize and destroy leukaemia cells is required, with the aim of reducing relapse rates. In previous years, dendritic cell (DC)-based immunotherapy has presented as a promising strategy for the elimination of MRD in patients with ALL (6-8).

DCs are professional antigen-presenting cells (APC), and are critical in the induction of cellular and humoral immunity (9). Various studies have reported that the injection of tumor antigen-loaded DCs induces tumor-specific cytotoxic T lymphocyte (CTL) responses and leukemia resistance (6,10-12). However, the primary obstacles to the introduction of this therapeutic strategy in clinical practice include insufficient numbers of DCs and insufficient production of cytokines. Therefore, DC vaccine therapy relies on either the generation of sufficient numbers of DCs, to prime CTLs, or administration of immunomodulatory agents to overcome deficiencies in DC and CTL function (5).

TA 1 Dosing

Has varied based on cancers studied and/or particular target (general immune response, CA, chronic infectious diseases)

1.6mg to 3.2mg SQ bi weekly

Alternately 400-600 mcg SQ daily

Consider consecutive daily dosing first 1-2 weeks then maintenance (biweekly)

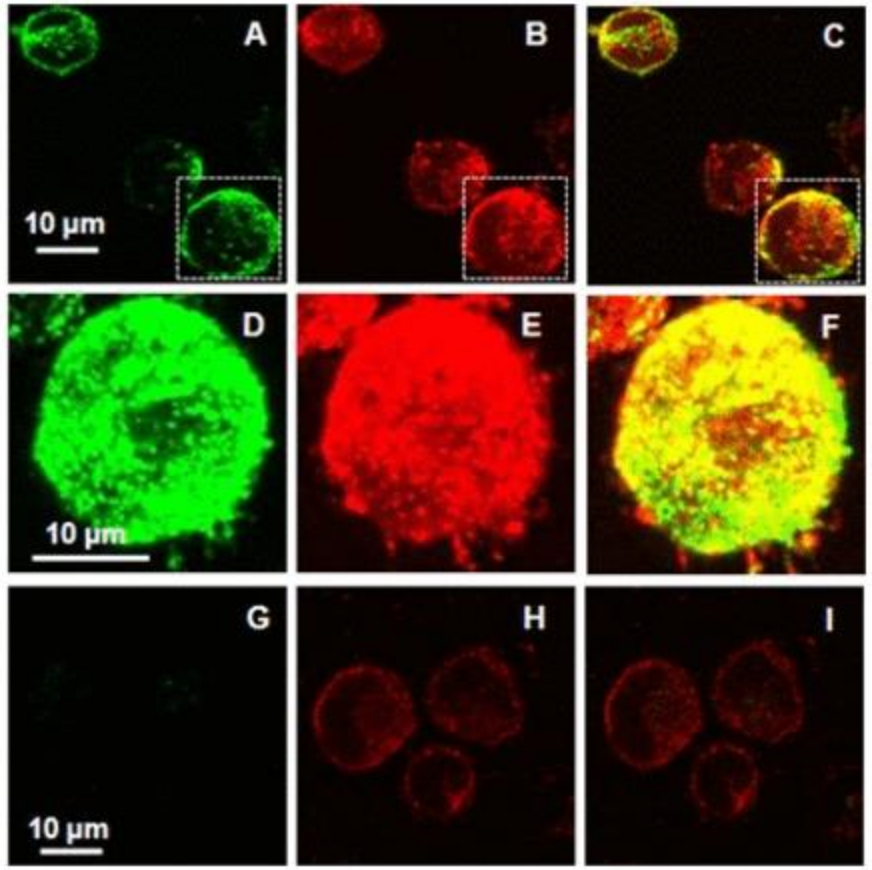
May calculate as 0.9mg/m2

Generally used as part of larger integrative treatment

PNC 27

PNC 27

- Membrane active peptide
- Binds to the HDM 2 protein expressed in cancer cell membranes of solid tissue tumors
- Induces transmembrane pore formation
- Results in tumor cell necrosis (p53 independent)
- Not clinically available in US



Confocal microscopy examination of the effect of PNC-27 on leukemia cells. The result of PNC-27 effect on cell membrane has been evaluated after 1 hour incubation of K562 cells with 25 µM peptide. The left panels of the figure (**A**, **D**, and **G**) show the representative images of cells labeled with DyLight® 488 bound to PNC-27 via anti-PNC-27 antibody; the central panels (**B**, **E**, and **H**) show the images of the same cells labeled with DyLight® 650 bound via anti-HDM-2 antibody to the surface HDM-2; the right panels of the figure are merged images of the green (PNC-27) and red (HDM-2) fluorescence. The resulting yellow photo (**F**) of merged green and red images indicates the close association of two proteins. The lower row presents single optical slice images of K562 cells mock treated with PNC-29 peptide. The resulting merged image of (**G**) and (**H**) is shown in (**I**) that shows no colocalization of the PNC-29 and HDM-2 peptides.

PNC 27 studies

Ann Clin Lab Sci. 2015 Fall;45(6):650-8.

Ex vivo Efficacy of Anti-Cancer Drug PNC-27 in the Treatment of Patient-Derived Epithelial Ovarian Cancer.

Sarafraz-Yazdi E[1], Gorelick C[2], Wagreich AR[3], Salame G[4], Angert M[5], Gartman CH[5], Gupta V[5], Bowne WB[6], Lee YC[4], Abulafia O[4], Pincus MR[7], Michl J[8].

Author information

Abstract

OBJECTIVE:

Despite an 80% response rate to chemotherapy, epithelial ovarian cancer has the highest case fatality rate of all gynecologic malignancies. Several studies have shown the efficiency of anticancer peptides PNC-27 and PNC-28 in killing a variety of cancer cells selectively in vitro and in vivo. The purpose of this study was to evaluate the efficacy of PNC-27 against human primary epithelial ovarian cancer.

METHODS:

We established primary cultures of freshly isolated epithelial ovarian cancer cells from patients with newly diagnosed ovarian cystadenocarcinomas. Two cell lines were obtained, one from mucinous cystadenocarcinoma, and the other from high-grade papillary serous carcinoma. The cancerous properties of these cells were characterized in vitro morphologically, by their growth requirements and serum independence. Treatment effects with PNC-27 were followed qualitatively by light microscopy, and quantitatively by measuring inhibition of cell growth using the MTT cell proliferation assay and direct cytotoxicity by measuring lactate dehydrogenase (LDH).

RESULTS:

PNC-27 inhibits in a dose-dependent manner the growth of and is cytotoxic to human primary cancer cells that had been freshly isolated from two ovarian epithelial cancers. The results further show that the control peptide PNC-29 has no effect on the primary cancer cells. Our results also show that PNC-27 is cytotoxic to cells from long-established and chemotherapy-resistant human ovarian cancer cell lines.

CONCLUSION:

These findings show, for the first time, the efficacy of PNC-27 on freshly isolated, primary human cancer cells. Our results indicate the potential of PNC-27 peptide as an efficient alternative treatment of previously untreated ovarian cancer as well as for ovarian cancers that have become resistant to present chemotherapies.

Ann Clin Lab Sci. 2014 Summer;44(3):241-8.

The anti-cancer peptide, PNC-27, induces tumor cell necrosis of a poorly differentiated non-solid tissue human leukemia cell line that depends on expression of HDM-2 in the plasma membrane of these cells.

Davitt K[1], Babcock BD[1], Fenelus M[2], Poon CK[2], Sarkar A[2], Trivigno V[2], Zolkind PA[2], Matthew SM[2], Grin'kina N[3], Orynbayeva Z[1], Shaikh MF[1], Adler V[1], Michl J[4], Sarafraz-Yazdi E[5], Pincus MR[6], Bowne WB[7].

Author information

Abstract

GOALS:

We have developed the anti-cancer peptide, PNC-27, which is a membrane-active peptide that binds to the HDM-2 protein expressed in the cancer cell membranes of solid tissue tumor cells and induces transmembrane pore formation in cancer, but not in normal cells, resulting in tumor cell necrosis that is independent of p53 activity in these cells. We now extend our study to non-solid tissue tumor cells, in this case, a primitive, possible stem cell human leukemia cell line (K562) that is also p53-homozygously deleted. Our purpose was twofold: to investigate if these cells likewise express HDM-2 in their plasma membranes and to determine if our anti-cancer peptide induces tumor cell necrosis in these non-solid tissue tumor cells in a manner that depends on the interaction between the peptide and membrane-bound HDM-2.

PROCEDURES:

The anti-cancer activity and mechanism of PNC-27, which carries a p53 aa12-26-leader sequence connected on its carboxyl terminal end to a trans-membrane-penetrating sequence or membrane residency peptide (MRP), was studied against p53-null K562 leukemia cells. Murine leukocytes were used as a non-cancer cell control. Necrosis was determined by measuring the lactate dehydrogenase (LDH) release and apoptosis was determined by the detection of Caspases 3 and 7. Membrane colocalization of PNC-27 with HDM-2 was analyzed microscopically using fluorescently labeled antibodies against HDM-2 and PNC-27 peptides.

RESULTS:

We found that K562 cells strongly express HDM-2 protein in their membranes and that PNC-27 co-localizes with this protein in the membranes of these cells. PNC-27, but not the negative control peptide PNC-29, is selectively cytotoxic to K562 cells, inducing nearly 100 percent cell killing with LDH release. In contrast, this peptide had no effect on the lymphocyte control cells.

CONCLUSIONS:

The results suggest that HDM-2 is expressed in the membranes of non-solid tissue tumor cells in addition to the membranes of solid tissue tumor cells. Since K-562 cells appear to be in the stem cell family, the results suggest that early developing tumor cells also express HDM-2 protein in their membranes. Since PNC-27 induces necrosis of K-562 leukemia cells and co-localizes with HDM-2 in the tumor cell membrane as an early event, we conclude that the association of PNC-27 with HDM-2 in the cancer cell membrane results in trans-membrane pore formation which results in cancer cell death, as previously discovered in a number of different solid tissue tumor cells. Since K562 cells lack p53 expression, these effects of PNC-27 on this leukemia cell line occur by a p53-independent pathway

GHK Peptide

GHK Peptide

- Tri Peptide (glycyl-L-Histidyl-L-Lysine)
- Extensively studied for both cosmetic and regenerative purposes
- Often present as GHK-Cu
- Has been shown to modulate gene expression relating to cancer

GHK known benefits

Tighten loose skin and reverse thinning of aged skin
Repair protective skin barrier proteins
Improve skin firmness, elasticity, and clarity
Reduce fine lines, depth of wrinkles, and improve structure of aged skin
Smooth rough skin
Reduce photodamage, mottled hyperpigmentation, skin spots and lesions
Improve overall skin appearance
Stimulate wound healing
Protect skin cells from UV radiation
Reduce inflammation and free radical damage
Increase hair growth and thickness, enlarge hair follicle size

GHK and cancer

GHK suppresses RNA production in 70% of 54 genes overexpressed in cancer patients

Affects gene regulation of multiple pathways

Resetting gene activity

Able to reactivate apoptosis system lost in cancer

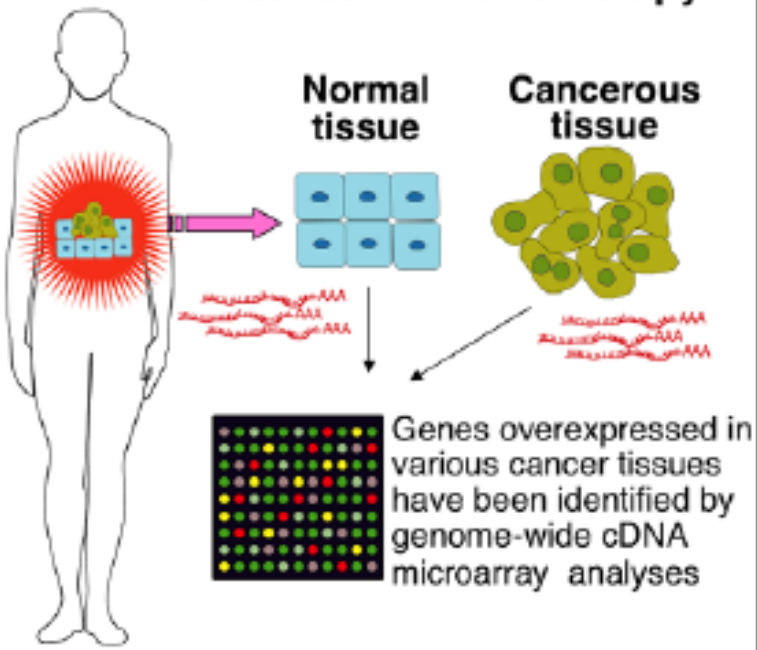
Dosing

- No established dose for CA Tx
- Clinical results observed with 2.5mg SQ 3 times weekly
- Safe, well tolerated
- Pleiotrophic effects may show improvements in other parameters (pain, inflammation)

Peptide vaccine therapy

- Used as Tumor Associated Antigens (TAAs)
- Overexpressed in cancerous tissues, not in healthy cells
- Highly immunogenic
- Currently in clinical trials

Identification of ideal tumor-associated antigens (TAAs) useful for cancer immunotherapy

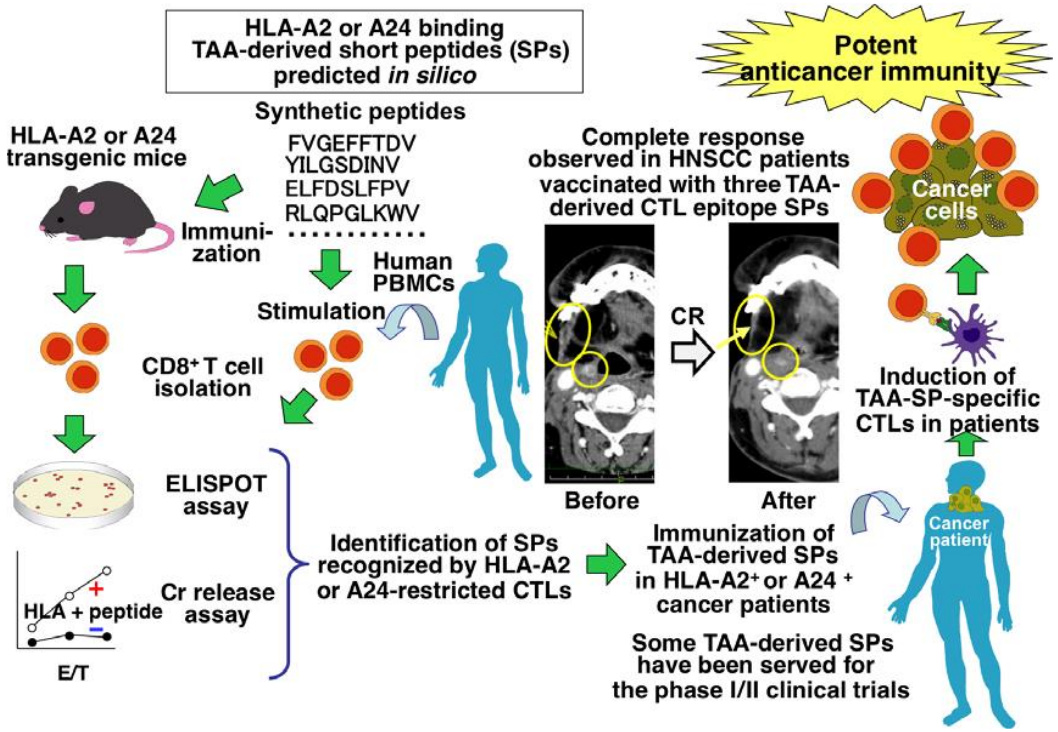


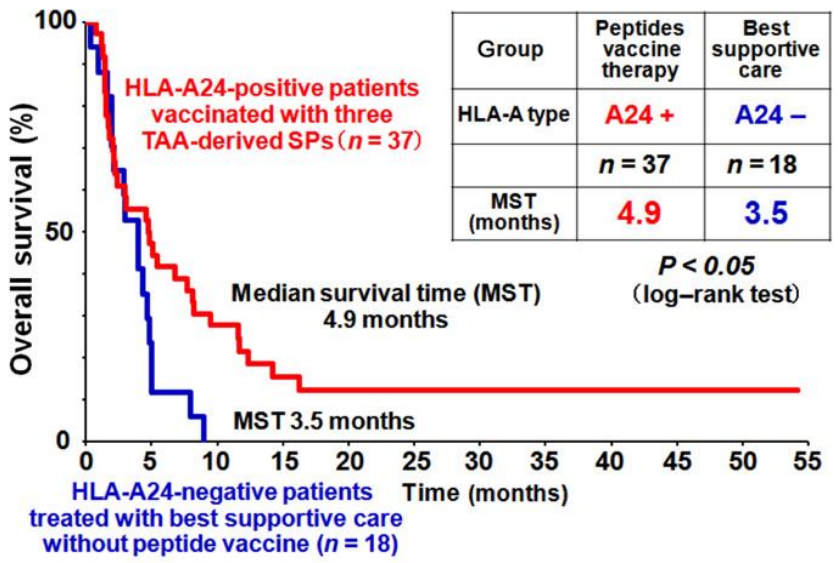
LY6K	CDCA1	IMP3
KIF20A	GPC3	FOXM1
CDH3	SPARC	CDC45L

These molecules are frequently over-expressed in cancerous tissues, fetal organs or testis but not in many other adult tissues

Organ-specific expression of TAAs

- **Oral and esophageal Ca.**
  LY6K, CDCA1, IMP3
- **Non small and small cell lung Ca.**
  LY6K, CDCA1, IMP3, KIF20A, FOXM1
- **Hepatocellular Ca.**
  GPC3
- **Cholangiocellular Ca.**
  CDCA1, IMP3, KIF20A, CDH3
- **Pancreatic Ca.**
  CDH3, FOXM1, KIF20A
- **Diffuse type gastric Ca.**
  SPARC
- **Urinary bladder Ca.**
  LY6K, CDCA1, FOXM1
- **Prostatic Ca.**
  IMP3
- **Osteosarcoma**
  IMP3


HLA-A2 or A24 binding
TAA-derived short peptides (SPs)
predicted *in silico*
Potent
anticancer immunity
Synthetic peptides
FVGEFFTDV
YILGSDINV
ELFDSLFPV
RLQPGLKWV
HLA-A2 or A24
transgenic mice
Immuni-
zation
Human
PBMCs
Stimulation
CD8+ T cell
isolation
Complete response
observed in HNSCC patients
vaccinated with three TAA-
derived CTL epitope SPs
CR
Before
After
Cancer
cells
Induction of
TAA-SP-specific
CTLs in patients
ELISPOT
assay
Cr release
assay
HLA + peptide
E/T
Identification of SPs
recognized by HLA-A2
or A24-restricted CTLs
Immunization of
TAA-derived SPs
in HLA-A2+ or A24 +
cancer patients
Cancer
patient
Some TAA-derived SPs
have been served for
the phase I/II clinical trials



Group	Peptides vaccine therapy	Best supportive care
HLA-A type	A24 +	A24 –
	n = 37	n = 18
MST (months)	4.9	3.5

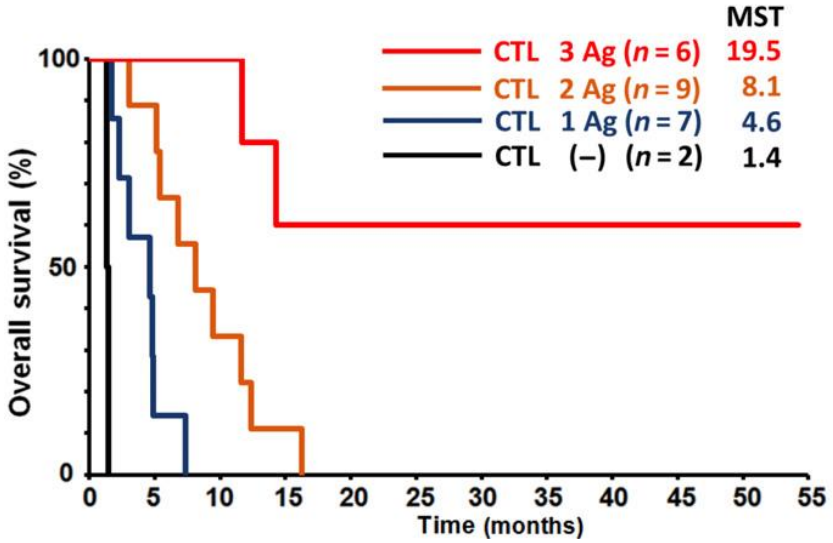


Table 1. Three tumor-associated antigen (TAA)-derived long peptides (LPs) identified to activate both T helper 1 (Th1) cells and CTLs

TAA-derived Th cell epitope LPs	Amino acids numbers	HLA class II alleles encoding for restriction molecules of Th cells	Cytokines produced	Presence of Th cells in patients vaccinated with SPs	Cross-presentation to CTLs	Restriction HLA-class I of CTLs specific to SPs included in LPs
LY6K (119–142) LP	24	*DRB1*08:03/15:02, DPB1*05:01*	Th1-type	+ ~ ++	NT	NT
LY6K (172–191) LP	20	*DRB1*15:02, DQ* (unknown)	Th1-type	++	Yes	A24
CDCA1 (55–78) LP	24	*DRB1*04:05/15:02, DPB1*02:01*	Th1-type	+++	Yes	A2/A24
CDCA1 (39–64) LP	26	*DRB1*09:01/15:02*	IFN-γ only tested	+++	Yes	A24
KIF20A (60–84) LP	25	*DRB1*15:02, DPB1*02:01*	Th1-type	+	Yes	A24
KIF20A (809–833) LP	25	*DRB1*15:02, DRB4*01:03* (*DR53*)	Th1-type	+	Yes	A2

We identified highly immunogenic TAA-derived 20–26-mer LPs that can activate Th1 cells restricted by HLA-class II molecules often found in Japanese populations in healthy donors and cancer patients.[47–49] Natural processing of TAA-LPs that can activate Th1 cells generated by stimulation with synthetic LPs, and cross-presentation to CTLs of short peptides (SPs) derived from LPs by dendritic cells were also proved in many LPs. Head and neck squamous cell carcinoma patients showed increased immune responses of TAA-LP-specific Th cells after vaccination with TAA-SPs. NT, not tested.

Integrating peptides into cancer management

- Regulatory
  - Off label vs investigational drug
- Avilability
  - Rx available from compounding pharmacies
- Integrating them into cancer treatment
  - Immune stimulation
  - Addressing chemo side effects
- Specific target points
  - Cancer type
  - Solid tumors
  - Mounting immune response

Continued

- Offered as complimentary therapy
- In conjunction with oncologist treatment and follow up
- May consider as last option in advanced patients
  - Improve QOL
  - Extend lifespan

- Ta 1, GHK and indirect OGF modulation seem as best approaches for immediate clinical use

Prophylactic Use: Why wait for cancer

- Immunosenescence progresses with aging
- Senescent cells contribute to increased cancer incidence
- Is there a role for preventive use of peptides in cancer reduction as part of comprehensive Age Management Intervention?
- Initial approach: Consider cyclic thymosin use and OGF modulation

Thymosin-alpha 1 (Zadaxin) enhances the immunogenicity of an adjuvated pandemic H1N1v influenza vaccine (Focetria) in hemodialyzed patients: a pilot study.

Carraro G[1], Naso A, Montomoli E, Gasparini R, Camerini R, Panatto D, Tineo MC, De Giorgi L, Piccirella S, Khadang B, Ceracchi M, De Rosa A.
Author information

Abstract

BACKGROUND:

Although influenza vaccination is widely recommended for immunosuppressed people, the same immune dysfunction that can increase the risk of contracting influenza might also compromise vaccine effectiveness, especially during pandemics. Clinical data have highlighted the role of adjuvants in improving vaccine efficacy. As uremic patients are especially vulnerable to infections, it is recommended that they should be vaccinated yearly against influenza. This paper presents the results of a pilot clinical trial, conducted in hemodialyzed patients with an adjuvated pandemic H1N1v influenza vaccine alone and combined with Thymosin-alpha 1.

METHODS:

Subjects were subdivided into 3 treatment groups receiving: the adjuvated pandemic influenza vaccine (Focetria) only (first treatment group), and the Vaccine+Thymosin alpha 1 (Zadaxin) at a dose of 3.2 and 6.4 mg (second and third treatment groups respectively). The immunoresponse was assessed on days 0, 21, 42, 84 and 168 after vaccine administration by means of Hemagglutination Inhibition (HI), Microneutralization (MN) and Single Radial Hemolysis (SRH) assays. The CHMP regards HI as the gold standard test to evaluate the immune response to influenza vaccines before influenza vaccines are licensed. The CHMP criteria are slightly different in adults (18-60-year-old subjects) and the elderly (>60 years old). Indeed, 40% of seroconversion, 70% of subjects seroprotected 21 days after vaccination, and a 2.5-fold increase in GMR (Geometric Mean Ratio) are required in adults, while in the elderly, the corresponding threshold values are: 30%, 60% and a 2-fold increase. All these criteria must be met for the licensing of a pandemic influenza vaccine. Safety evaluation was performed by means of Adverse Event (AE) recording, laboratory assays (hematology and chemistry), electrocardiogram, and assessment of vital signs.

RESULTS:

Three populations were considered: Intention-To-Treat (ITT) (94 patients), Per Protocol (PP) (82 patients), and Safety population (99 patients). With regard to the Geometric Mean Titer (GMT) and the Geometric Mean Ratio (GMR) of HI on Day 21 in the ITT population, both "Vaccine+Thymosin alpha 1" groups presented better results than the "Vaccine only" group. A large proportion of ITT patients in the two Vaccine+Thymosin alpha 1 groups achieved seroconversion by Day 21. On Day 42, the decrease in the GMT of HI was greater in the Vaccine+Thymosin alpha 1 groups than in the vaccine only group. Similar results were obtained in the PP population. The CHMP criteria were fully met in the groups treated with Vaccine+Thymosin alpha 1. No AE was found to be related to Thymosin alpha 1 nor to the Focetria vaccine.

CONCLUSIONS:

Although further studies in larger hemodialyzed populations are necessary**, it can be concluded that Thymosin alpha 1 enhanced the immunogenicity of the pandemic influenza vaccine used. Moreover, it proved safe and well tolerated, and did not affect hematology or blood-chemistry values.**

[The prophylactic effect of thymosin alpha 1 on the acute exacerbation of chronic obstructive pulmonary disease].

[Zheng BX[1], Cheng DY, Xu G, Fan LL, Yang Y, Yang W.

Author information

Abstract

OBJECTIVES:

To investigate the prophylactic effect of thymosin alpha 1 and its mechanism on patients with chronic obstructive pulmonary disease.

METHODS:

Eighty patients with chronic obstructive pulmonary disease were divided into two groups. In the treatment group, 42 patients received thymosin alpha 1 1.6 mg hypodermic injection, quague die alterna, for 10 times. All patients were followed for 6 months, and were assessed the number and days of patients with acute exacerbation at 3 and 6 months. In two groups, before treatment and 3 and 6 months after treatment, the pulmonary function tests were measured, and the blood samples were collected for the measurement of the blood IgA, IgG, IgM, CD3, CD4 and CD8 levels.

RESULTS:

In the treatment group, the number and days of patients with acute exacerbation were significantly lower in comparison with those of the control group. After treatment of thymosin alpha 1, blood CD4 and CD4/CD8 levels were significantly increased.

CONCLUSION:

Thymosin alpha 1 has a good protection for the acute exacerbation of chronic obstructive pulmonary disease, by incresing body cellular immune activity

Case examples

- 72 y/o male patient, diagnosed with Stage 3b B cell lymphoma
- Received 2 infusions of MetEnk 1 week apart and TA1 1mg SQ x 10 days prior to starting chemotherapy
- Then continued on TA1 sq 1.6mg twice/week while on chemotherapy
- By end of second month labs had normalized (LDH, WBCs)
- By 4th month, no sign of tumors
- Oncologist told patient he was the fastest responder for tumor type/stage he has had in 30 years

Case #2

- 80 y/o male patient presented to the clinic with stage 4 Colon CA
- Multiple nodules in lungs and liver
- Told by oncologist that nothing else could be done- given 3 months to live
- TA1 given 1mg x 10 days; then 1.6 mg twice a week
- Metenk infusions (15mg total per infusion- twice a week for 8 weeks)
- GHK SQ 2.5mg 3 x week for first 3 months, then 2.5 mg SQ weekly
- Achieved partial response (PR; RECIST) after 6 months and 3 years later has maintained SD; completely asymptomatic and leads normal life

Conclusions

- Peptides, both endogenous and synthetic, play a role in both immune health and as complementary therapies in cancer treatment
- Multiple therapeutic based peptide modalities are currently in research and development
- Clinically available peptide therapies have shown an excellent safety profile
- Results require ongoing treatment in most cases
- Integrate, Integrate, Integrate
- Consider as part of a proactive Age Management Medicine Program