INSTITUT PASTEUR — UNIVERSITE PIERRE & MARIE CURIE

The 2nd PSU International Teaching Platform on Tumour Immunology and Immunotherapy

Jointly organized by
Prince of Songkla University, Université Pierre et Marie Curie (Paris 6) and Institut Pasteur

December 15 – 20, 2003
At The Department of Biomedical Sciences
Faculty of Medicine, Prince of Songkla University,
Hat Yai, Songkhla, Thailand

Lecture 8:
Cancer vaccination
Prof. Eric Tartour

December 17, 2003

CANCER VACCINES

1) Arguments supporting the concept of immunosurveillance leading to the development of cancer vaccine to boost the immune system
2) Cancer vaccine based on antibody induction
   - Preventive vaccine against virus
   - Vaccine against melanoma antigen
3) Cancer vaccines based on the induction of T lymphocytes (CTL)
   - Peptides (Heteroclitic Immunization, adjuvant…)
   - Polyepitopic vaccine: Vectorization by viruses or non replicative vectors
4) Use of Dendritic cells
   - Rational
   - Clinical results
   - Optimization
5) Cancer vaccine and risk of auto-immunity
6) Potential mechanisms leading to tumour escape from immune recognition

2nd PSU Workshop on Tumour Immunology and Immunotherapy, December 15-20, 2003. Prince of Songkla University, Université Pierre et Marie Curie and Institut Pasteur

Arguments supporting the concept of immunosurveillance leading to the development of cancer vaccine to boost the immune system

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Epidemiology and cancer

- Increase in the frequency of some cancers (Kaposi sarcoma, EBV associated lymphomas, cervix carcinomas...) in immunodepressed patients. :
  . Congenital immunodeficiency.
  . Acquired immunodeficiency - AIDS,
  - Immunosuppressors

- Graft Versus Leukemia (GVL) during allogeneic bone marrow transplantation.

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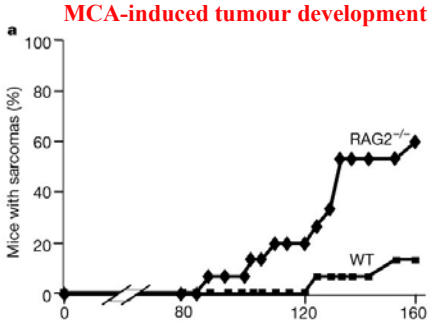
Lymphocyte-deficient mice are highly susceptible to MCA-induced tumour development



Shankaran et al. Nature 2001

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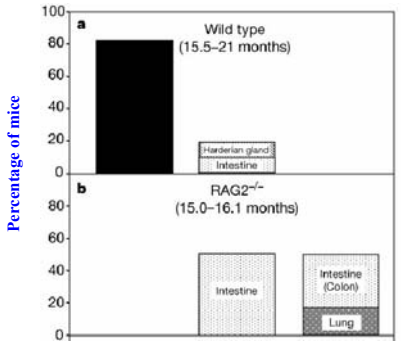
Increased development of spontaneous neoplastic disease in immunodeficient mice.



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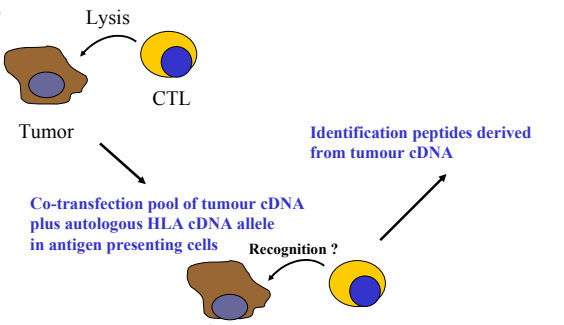
METHODS FOR THE IDENTIFICATION OF TUMOR ANTIGENS

1)



SEREX Technique

Screening phages or bacteria transfected with a cDNA library derived from tumours with sera of cancer patients.

- Sera are easier to obtain than tumour specific CD4 T or CD8 T lymphocytes.

- Many antigens were characterized during these last years using this technique.
  e.g.: NY-ESO
  (http://www.licr.org/SEREX.html).

Tumour-associated antigens

A/ Peptides derived from antigens recognized by CD8 T cells

1. Melanoma-melanocyte differentiation antigen
- Mart-1 (Melan A)
- Gp100 (pmel-17)
- Tyrosinase
- TRP1 (gp75)
- TRP2
- MSH- R

2. Cancer-Testis antigen
- Mage 1, Mage 2, Mage 3, Mage 12
- Bage, Gage, Rage
- NY-ESO-1
- N-acetylglucosaminyltransferase V (intronic peptide)

3. Mutated antigens
- β catenine
- CDK-4
- Caspase-8
- KIA0205
- HLA-A2
- ras
- p53

4. Antigens present in normal tissue but over-expressed in tumours
- G-250
- Her-2/neu
- p53
- Telomerase catalytic protein
- ACE
- α foeto-protein (αFP)

B/ peptides derived from antigens recognized by CD4 T cells

1. Peptides derived from non mutated antigens (cancer testis or Melanoma-melanocyte differentiation antigens)
- gp100
- Mage 1
- Mage 3
- Tyrosinase
- NY-ESO-1

2. Peptides derived from mutated antigens
- Triosephosphate isomerase
- CDC-27
- LDLR-FUT

VIRUS	TUMOURS	Other symptoms associated with viral infections
EBV	- Burkitt Lymphomas - Cavum carcinomas - Hodgkin lymphomas	Infectious mononucleosis Hemophagocytosis syndrome. Immunodeficiency (Purtillo syndrome)
HTLV1	- T -Leukemias	Spasmodic paralysis syndrome
HPV16,18	- Cervix carcinomas	Cervical intraneoplasia laryngeal papillomatosis
HPV1-45	- Bowen disease (In situ carcinoma) - Squamous-cell carcinomas (immunodepressed patiens)	Dyskeratosis , Wart
HBV / HCV	- Hepatocarcinoma	Hepatitis, Cirrhosis
KSHV (HHV8)	- Kaposi Sarcomas	Castleman disease

Natural humoral and cell-mediated immunity against cancer

1 Humoral response

- Antibodies against many tumor antigens (p53, HER2/neu, Muc 1, GD2, NY-ESO1, HU…) in the serum of cancer patients.

2 CD4 T cells and CD8 T cell against tumour peptides in the blood or TIL of cancer patients.

Cancer-testis antigen (Mage family (T Boon), **Mage A10, NY-ESO).**
Melanoma differentiation antigens: Melan A, Tyrosinase and gp100
Self antigens: Muc 1, HER-2/neu, Proteinase 3, survivin
Virus.

Cancer vaccine based on antibody induction

- Preventive vaccine against virus
- Vaccine against melanoma antigen

Efficacy Analyses of a Human Papillomavirus Type 16 (HPV-16) L1 Virus-like–Particle Vaccine.

	HPV vaccine	Placebo
Persistent HPV-16 infection	0/768	41/765
Cervical intraepithelia neoplasia	0	9

Median follow-up: 18 months

Koutsky LA . N Engl J Med 2002

Efficacy Analyses of a Human Papillomavirus Type 16 (HPV-16) L1 Virus-like–Particle Vaccine.

Immunogenicity Analysis

After the third dose (month 7), the geometric mean titer of HPV-16 antibodies was 1510 mMU per milliliter among the 619 women who received HPV-16 vaccine and less than 6 mMU per milliliter among the 631 women who received placebo.

For reference, the geometric mean titer of HPV-16 antibodies was 25.7 mMU per milliliter at enrollment among 337 women who had detectable HPV-16 antibodies on day 0.

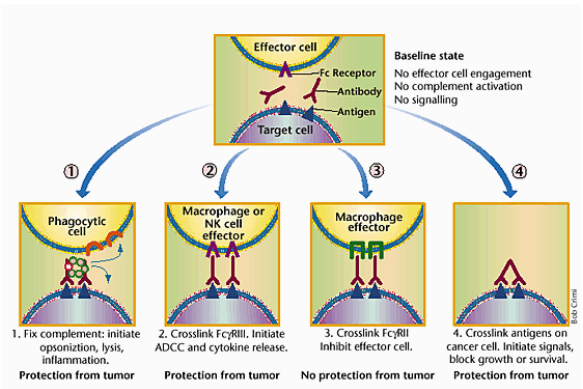
TABLE 4. INCIDENCE OF LIVER CANCER PER 100,000 CHILDREN IN BIRTH COHORTS DETERMINED ACCORDING TO THE DATE OF IMPLEMENTATION OF THE HEPATITIS B VACCINATION PROGRAM.

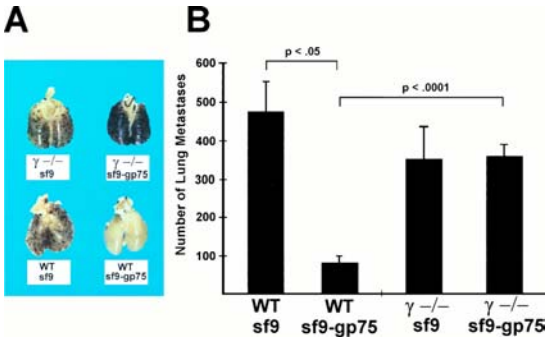
AGE AT DIAGNOSIS (YR)	BEFORE-PROGRAM COHORT (JULY 1974–JUNE 1984)		AFTER-PROGRAM COHORT (JULY 1984–JUNE 1986)	
	POPULATION	NO. OF CANCERS (INCIDENCE)	POPULATION	NO. OF CANCERS (INCIDENCE)
6	3,940,747	18 (0.46)	648,642	0 (0.00)
7	3,938,119	21 (0.53)	647,051	1 (0.15)
8	3,931,983	19 (0.48)	644,892	2 (0.31)
9	3,928,721	24 (0.61)	340,521*	0 (0.00)
Total	15,739,570	82 (0.52)	2,281,106	3 (0.13)†

*This value is based on data for the cohort born from July 1984 to June 1985.

†P<0.001 for the comparisons between birth cohorts.

Chang MH N Engl J Med 1997





Active protection from melanoma metastases requires FcRs

Clynes R. PNAS 1998

Monoclonal antibodies in the treatment of cancers in human

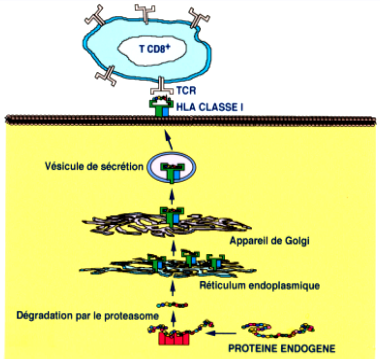
	Target	Indications
Trastuzumab* Herceptin	HER2/neu	Breast Cancer
Rituximab* (Chimeric Abs)	CD20	Follicular Lymphoma B-CLL
Alemtuzumab* (Campath-1H) Humanized Abs	CD52 (Malignant B and T cells)	B-CLL Sezary syndrome

Cancer vaccines based on the induction of T lymphocytes (Cytotoxic T lymphocytes (CTL))

ROLE OF CYTOTOXIC T LYMPHOCYTES IN THE CONTROL OF TUMOR DEVELOPMENT

- Clinical responses after administration of CTL and TIL in adoptive immunotherapy protocols.
- Identification of CTL in biopsies of cancer lesions derived from spontaneous regressing tumours.
- Correlation in murine models between the ability to elicit CTL and anti-tumour clinical responses.

Antigen presentation restricted by HLA class I molecules



In spite of some rare studies in which peptide vaccine were shown to elicit CTL (Feltkamp Eur J Immunol 1995, Cormier JN. Cancer J. Sci Am 1997, Pass HA. Cancer J. Sci Am 1998) most cancer vaccines based on peptide immunization failed to induce efficient anti-tumour CTL. (Marchand.Int J. Cancer 1999, Lewis JJ. Int J. Cancer 2000)

To improve the peptide immunization efficiency different groups proposed to:

- Increase the binding of peptide to HLA molecule by introducing mutations in wild type tumour peptides which will enhance the affinity of peptide to HLA molecules. This more stable HLA-peptide complex should lead to better induction of CTL…without changing the recognition of the wild type peptide by the induced CTL.

Peptides derived from glycoprotein $B_{498-505}$ from herpes simplex virus.

SEI: SEIEFARL (Natural peptide) **SSI**: SSIEFARL

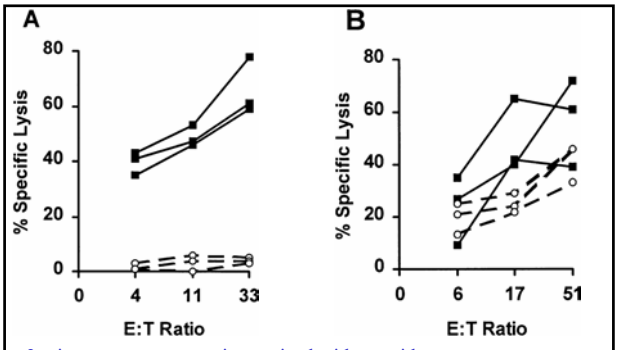
The aminoacid in position 2 of the SEI and SSI peptides bind to the aminoacid 24 of the K^b molecule

B6 mice: H-2K^b

B6 C-H-2^{bm8} mice: H-2K^{bm8} → aminoacid 24 of the K^b molecule is mutated

SEI binds poorly to H-2K^b; SSI binds strongly to H-2K^b

SEI et SSI bind strongly to the H-2K^{bm8}

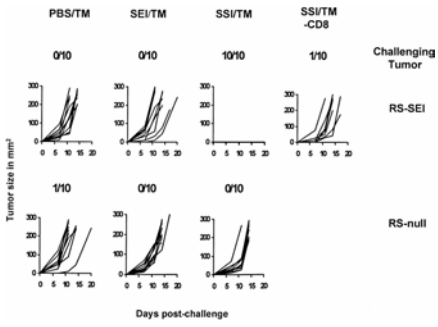


3 mice per groups were immunized with peptide SSI ■ or SEI ◯

A B6 mice (H-2K^b)

B B6 C-H-2^{bm8} (H-2K^{bm8}) mice

Dyall et al. J. Exp. Med 1998



Dyall et al. J. Exp. Med 1998

In vivo ability of the heteroclitic vaccine SSI to protect mice against a transplantable tumour expressing SEI.

Rosenberg SA. Nature Med 1998.

- gp100 $_{209-217}$ natural peptide which binds to intermediate affinity to HLA-A2

- Mutation of the peptide in AA position 2 (AA2 T → M) = gp100 $_{209-2M}$

8 melanoma patients were immunized with natural peptide:
gp100 $_{209-217}$ + IFA: 2 T cell response (CD8) (25%) and no clinical response

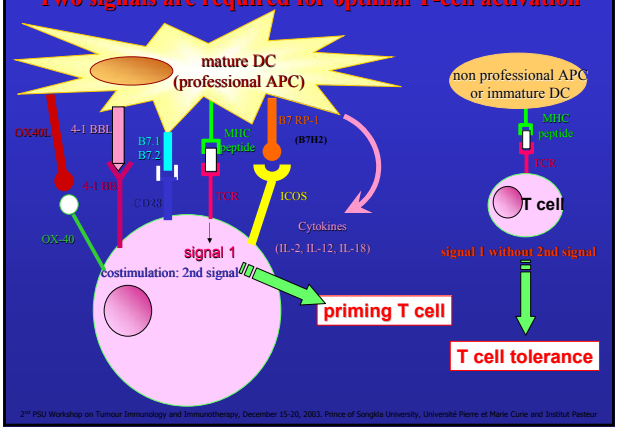
19 melanoma patients were immunized with modified peptide
gp100$_{209-2M}$ + IFA + IL-2: 3 T cell response (CD8) (15%) associated with **clinical response in 42% of patients.**

To improve the peptide immunization efficiency different groups proposed to:

- Increase the binding of peptide to HLA molecule by introducing mutations in wild type tumour peptides which will enhance the affinity of peptide to HLA molecules. This more stable HLA-peptide complex should lead to better induction of CTL…without changing the recognition of the wild type peptide by the induced CTL.

- Peptide will not target antigen presenting cells and in the absence of second signal, this peptide vaccination may lead to tolerance.

Two signals are required for optimal T-cell activation



ADJUVANT

Use of adjuvants which recruit and/or activate antigen presenting cells.

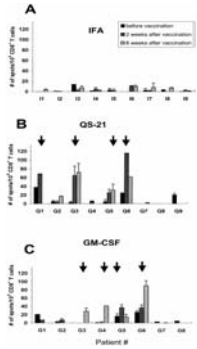
- **GM-CSF**
- **Anti-CD40**
- **CpG**
- **IFNα**

GM-CSF

- **In mice, GM-CSF cDNA transfection into tumours increased the immunogenicity of the tumours (Dranoff et al. PNAS. 1993)**

- **In human, immunisation of HLA-A2 melanoma patients with Mart 1, tyrosinase and gp100 peptides during three cycles. The addition of GM-CSF during the 4th cycles increased CTL response against these peptides. (Jager E. Int J. Cancer 1996)**

T-cell responses against tyrosinase 368-376(370D) peptide in HLA*A0201+ melanoma patients: randomized trial comparing incomplete Freund's adjuvant, granulocyte macrophage colony-stimulating factor, and QS-21 as immunological adjuvants.



Schaed G Clin Cancer Res 2002

Limits in the development of CD8 peptide based cancer vaccines

- Accumulative evidences also suggest that tumour cells could easily escape to immune attacks induced by peptide by means of partial or total loss of the expression of tumour antigen and MHC class I molecules.

- The use of peptides is also restricted to patients with a particular type of HLA and is not applicable to large outbred population.

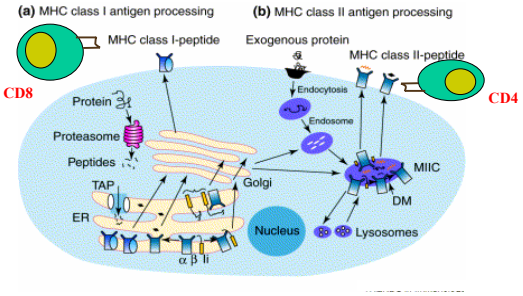
- Polypeptides may allow the activation of CD4 T helper cells which play a critical role in initiating immune response and in the priming and differentiation of $CD8^+$ T cells

- In some tumour models, CD4+T cells are the main effector cells responsible for the induction of anti-tumour immunity

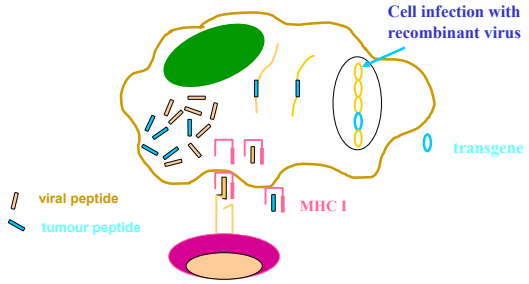
- **Unfortunately, recombinant proteins is efficient to elicit humoral responses but failed to induce specific CD8-T lymphocytes.**

- **Inefficiency of cross presentation may explain this failure.**

Knowledge in the antigen presentation pathways led to the concept that antigen needs to be vectorized to increase its immunogenicity.

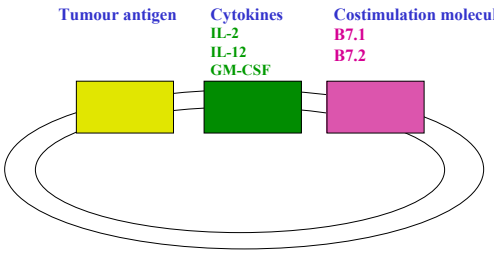


Viruses have the ability to deliver exogenous antigens in the cytosol of the cells



CD8 Cytotoxic T cells

Design of anti-cancer vaccines



Virus: Vaccinia virus, Adenovirus, Retrovirus…

Overview of cancer vaccine protocols based on the use of recombinant viruses.

Vaccinia-E6-E7 HPV16 et 18 **Cervix carcinoma**: .	1 CTL out 3 patients treated	Borysiewicz LK. Lancet 1996
Retrovirus p53 **Non small cell lung carcinoma**	3 local regression out 9 injected tumors	Roth JA. Nature Med 1996
ADV-Mart 1 **mélanoma**	1CR/16 patients 5/23 CTL	Rosenberg JNCI 1998
ADV-gp100 **mélanoma**	No PR no CR 0/16 CTL	Rosenberg JNCI 1998
Vaccine-Muc1 **breast cancer**	2/9 CTL	Scholl S J. Immunother 2000
Alvac-CEA **adénocarcinoma**	No PR no CR CTL 7/9	Marshall JL. J. Clin Oncol 1999

Problems encountered with the use of recombinant virus

- Several safety issues have not been conclusively resolved. For example, reversion of attenuated live vectors to virulent strains by genetic recombination cannot be excluded.

- Even attenuated viral or bacterial strains are associated with health risks for immunodeficient recipients.

- The immune response to virus proteins is a significant limitation to successful immunization in animal and human

Synthetic vectors which target exogenous antigens into the MHC class I pathway

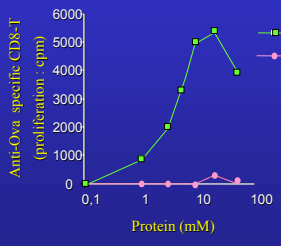
1- Heat Shock Proteins (HSP) GRP94 (gp96), HSP70

2- Toxins
- Adenylate cyclase toxin (CyaA) from *Bordetella pertussis*
- Pertussis toxin from *Bordetella pertussis*
- Lethal Factor from *Bacillus anthracis*
- Shiga toxin from *Shigella dysenteriae*
- Pseudomonas exotoxin A from *Pseudomonas aeruginosa*

3- Virus Like Particles

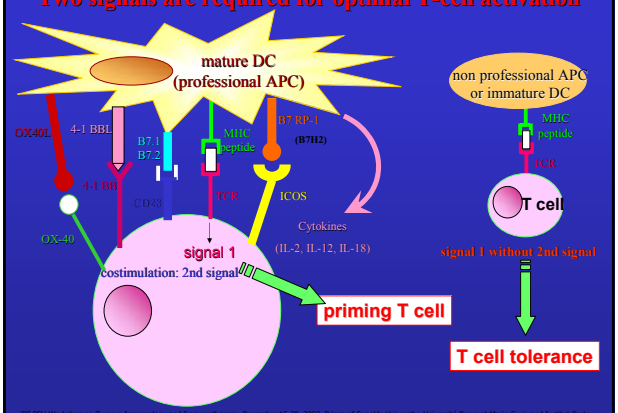
4- Other transduction proteins
- Tat (HIV)
- Outer membrane protein A from *Klebsiella*
- Homeodomain from *Antennapedia* (AntpHD)

Targeting exogenous antigens in the MHC class I pathway does not always allow the induction of CTL in mice.



Tat and the Pseudomonas exotoxin A target exogenous antigen into the MHC class I pathway but did not elicit CTL *in vivo*.

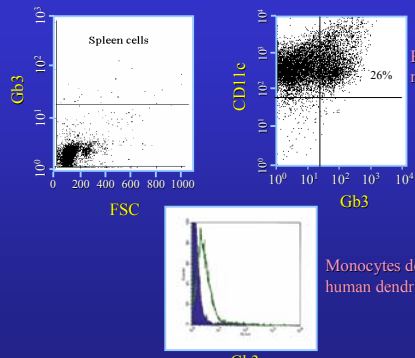
Two signals are required for optimal T-cell activation



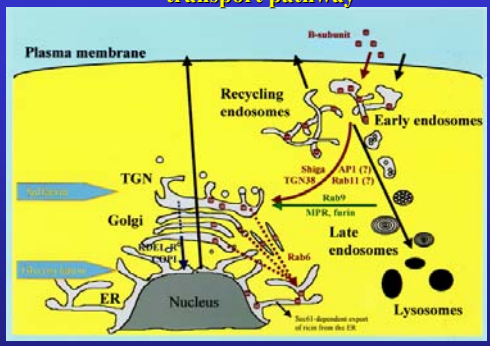
Rationale for the design of a synthetic vector to be developed in cancer vaccines.

- Ability to target exogenous antigen into the MHC class I pathway.
- Preferential delivery of antigen in professional antigen presenting cells (dendritic cells).
- Role in the activation and maturation of dendritic cells.

Gb3 is preferentially expressed on dendritic cells in mice and human

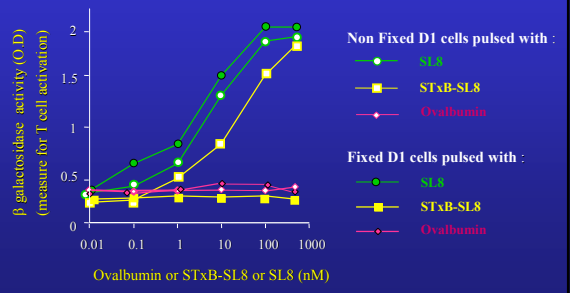


The Shiga toxin follows an intracellular retrograde transport pathway

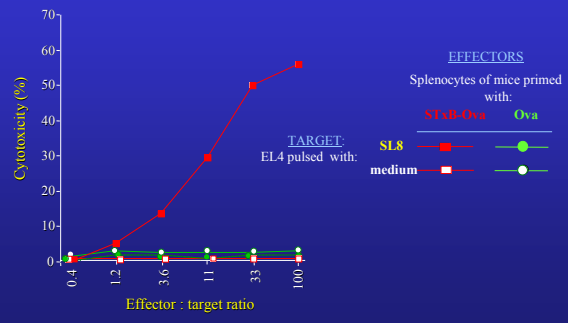


Johannes L and Goud B. *Traffic*, 2000.

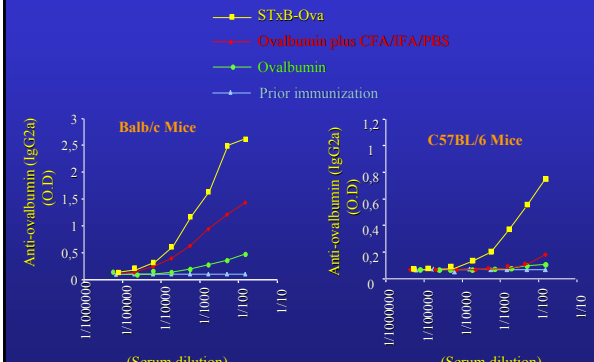
Dendritic cells present CD8 epitopes derived from Shiga-SL8 fusion proteins.



Shiga B subunit coupled to whole protein elicits specific cytotoxic T lymphocytes



STxB increases anti-ovalbumin IgG 2a antibodies



Characteristics of Shiga toxin as a synthetic vector

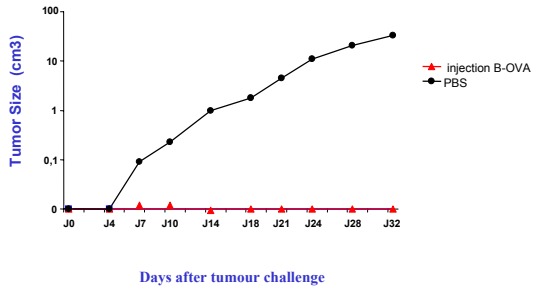
. Targets dendritic cells in mice and human.

. Delivers exogenous antigen into both the classical MHC class I and class II pathway

. Stimulation of CD4 specific T cells with a dominant TH1 polarization

. Induction of CTL against different antigens (P815 A, ovalbumin…) without adjuvant

Vaccination with B-Ova protects against a challenge with Ova-expressing tumour (EG7)

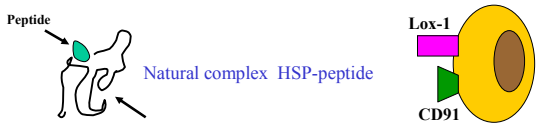


Comparative features of toxins and related molecules as vectors for cancer vaccines

	Anthrax toxin (Modified Lethal Factor)	Shiga toxin (B subunit)	Bordetella pertussis (Adenylate cyclase)	Kp OmpA
Ability to deliver whole protein	+	+	+	+
Induction of CTL in mice without adjuvant	+	+	+	+
Activation of specific CD4 TH1 cells	+	+	+	?
Preferential targeting of dendritic cells	?	+ Gb3	+ CD11b	+ TLR2
Tumour protection	?	+	+	+

Heat Shock Protein (Gp96/grp94, HSP70, HSP90)

Vector and natural adjuvant of immunity



- HSPs are highly conserved proteins that serve as chaperones for newly synthesized proteins
- HSPs binds preferentially to Antigen Presenting Cells (Lox-1, CD91)
- HSPs facilitate uptake and cross-presentation of the peptides by antigen-presenting cells

HSP = **Antigenic fingerprint of the cells**.

- Vaccination with autologous tumour-derived HSP-peptide complexes has been shown to elicit CTL against peptides complexes with HSP (Srivastava PK. Nat Rev Immunol 2002)

- Vaccination with autologous tumour-derived HSP-peptide complexes also results in both prophylactic and therapeutic antitumor activity in multiple animal tumor models (Tamura Y Science 1997)

Human clinical protocols developed with HSPs

Janetzki S: - gp96 prepared from autologous tumours
- Induction CTL against autologous tumours in 6/12 patients (**Int J. Cancer 2000**).

Amato R: - gp96 purified from autologous renal carcinomas - 1CR et 3PR among 16 treated patients (**ASCO 2000**).

Belli F: In five out of 17 patients, T-cell reactivity increased against autologous melanoma after immunization (**J Clin Oncol 2002**).

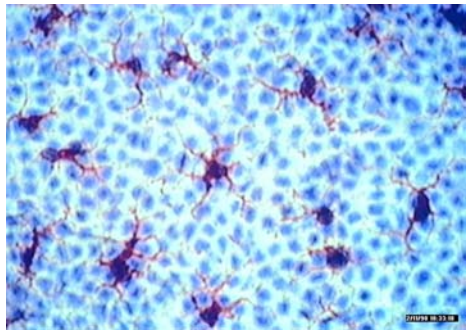
- Two of the 28 patients treated with measurable disease had complete response in multiple, small soft tissue metastases while receiving the vaccine

Limits in the use of HSPs

- Requirement of autologous tumours. Inefficiency of allogeneic HSPs in vaccine protocols.

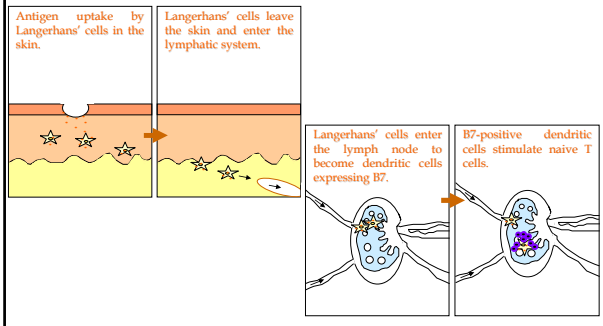
- Purification of HSP is tedious and time consuming.

- Of the 64 patients who had tumour collected, 40% could not receive the initial four weekly injections (e.g., one injection per week for 4 weeks), mainly because not enough vaccine could be made or because their melanoma progressed while vaccine was being prepared (Belli F J Clin Oncol 2002)

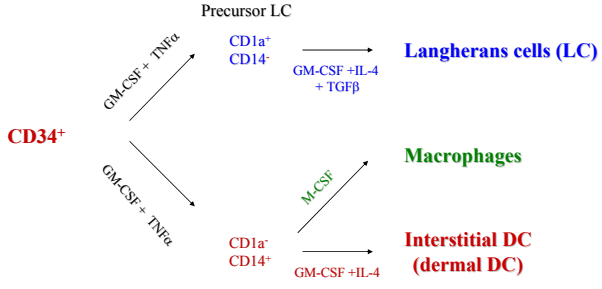


Dendritic cell network in epidermis

Langerhans' cells can take up antigen in the skin and migrate to lymphoid organs where they present it to T cells.



ORIGIN AND DIFFERENTIATION OF MYELOID DENDRITIC CELLS



Different steps of the immune response involving dendritic cells

- Antigen internalization

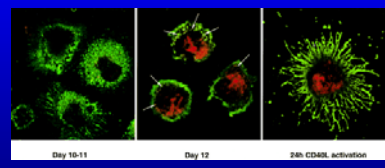
- Migration of dendritic cells to lymph nodes which is associated with their maturation.

- Activation and polarization of T and B lymphocytes in lymph nodes

Analysis by confocal microscopy of the subcellular localization of DC-LAMP during the maturation of $CD34^+$ derived DC.

HLA DR (green)

DC-LAMP (red)



Immature DC | Intermediate | Fully mature DC

Signals required for the maturation of immature human DC

- Ligation of CD40
T cells or anti-CD40 mAb or cells transfected with CD40L (CD154)

- Pathogen derived signals
LPS, double strand RNA, bacteria or bacterial products (SAC, LTA of BG^+, LAM of mycobacteria), immunostimulatory unmethylated CpG oligonucleotides, poly (I:C)

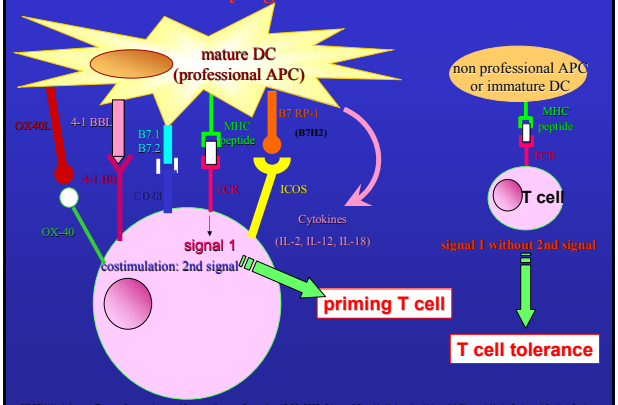
- Cytokines
PGE2, IFNα, TNFα and IL-1β or TNFα alone

- Cell death by necrosis (not apoptosis)

Phenomenon associated with maturation changes of DC

- Increase in the expression of T cell costimulatory molecules like CD86 and CD40
- Increase in the capacity to produce IL-12
- Increase in the capacity to resist to immunosuppression by IL-10
- Development of new repertoire of chemokine receptors, especially CCR7
- Production of DC survival molecules like TRANCE-R (= RANK)
- Redistribution of MHC class II molecules from lysosomes to the cell surface.
- Increase of subunits associated with immunoproteasomes and PA28
- Decreased of phagocytic receptors: αvβ5 integrin for apoptotic bodies and FcγR for immune complex.

Role of costimultory signals in the activation of T cells



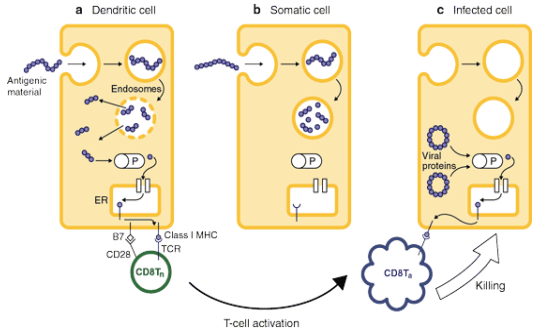
Arguments for using dendritic cells in cancer vaccines

- Cells specialized in the presentation of antigens to T cells.

- Mature dendritic cells produce cytokines favouring TH1 polarization of T cells (IL-2, IL-12, IL-18).

- Dendritic cells have the ability to cross-present antigens.

Ability of dendritic cells to target exogenous antigen in the cytosol



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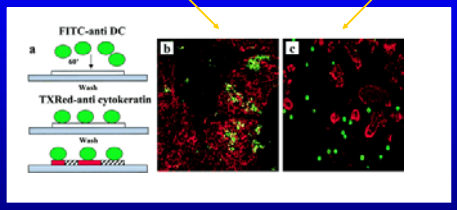
- Dendritic cells have the ability to cross-present antigens.

- Host dendritic cells are blocked in an immature state in tumor microenvironment.

Only immature dendritic cells are found in close contact with tumours

Immature DC (green) bind to tumour cells (red)

Mature DC (green) don't bind to tumour cells (red)



(Bell et al 2000)

Mature DC were virtually absent in liver tissue from patients with hepatocellular carcinoma (Chen S et al. 2000)

Environmental factors and local cytokine production in the tumor microenvironment inhibit DC maturation.

- PGE2 affects early development of tissue type immature DCs, inducing IL-12 deficient cells with a TH2 promoting function.

- IL-10 inhibits both the ability of DCs to produce IL-12 and their stimulatory capacity. IL-10-exposed DCs have a residual TH2-driving function.
IL-10 treated DC are able to induce tolerance in mice and anergy in human T cells.

- IL-6 , M-CSF and VEGF inhibits the maturation of DC.

Clinical anti-tumour response after vaccination of mice with dendritic cells pulsed with tumour antigens

TUMOURS	METHODS TO LOAD DC	CLINICAL RESPONSES	AUTHORS
- 3 LL (lung carcinoma)	Mut 1 (peptides)	Prevention of tumour growth	Mayordomo JI 1995
- C3-HPV 16	HPV 16 E7 (peptide)	Treatment of established tumour	
- 3T3-p53 fibroblastes transfected with mutated P53	Mutated P53 (peptides)	Treatment of established tumour	Gabrilovich DI 1996
- MCA-205 fibrosarcoma - TS/A (breast carcinoma)	Elution of peptides	Treatment of established tumour	Zitvogel L. 1996

Clinical anti-tumour response after vaccination of mice with dendritic cells pulsed with tumour antigens

Tumours	Methods to load DC	Clinical responses	Authors
NFSA-Mart1 (Fibrosarcoma transfected with Mart 1)	Mart 1 (Adenovirus)	Prevention of tumour growth Treatment of established tumour	Ribas A. 1997
MC-38-Muc 1 (Adenocarcinoma Transfected with Muc 1)	Muc 1 (Adenovirus)	Prevention of tumour growth	Gong J. 1997
B16 Melanoma	Tumour lysate	Treatment of established tumour	Shimizu K 2001
MC38 (Adenocarcinoma)	Fusion tumour-DC	Treatment of established metastases	Gong J. 1997

16 MELANOMA METASTATIC PATIENTS

↓

Dendritic cells pulsed *ex vivo* with peptides or tumour lysates

↓

ADMINISTRATION IN LYMPH NODE

- No Toxicity
- CTL + HSR to tumour antigens in 11/16 patients.
- 5 Clinical responses (2CR ET 3PR) even in metastatic sites.

F.O. Nestle . Nature Medecine 1998

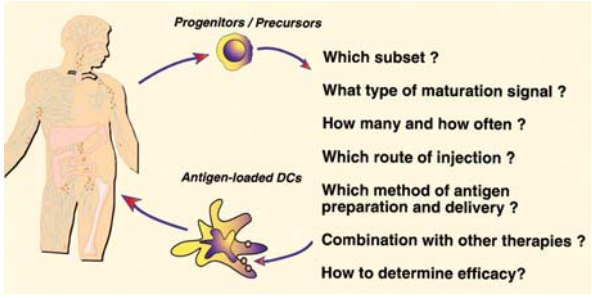
Human Clinical trials with DC

Murphy, Pacific Northwest Cancer foundation
Patients with **prostate cancer** (n = 60)
6 i.v injections (once every six weeks) of $2x10^7$ DCs pulsed with PMSA
25-30% overall response rate

Schuler G., Department of Dermatology, Erlangen, Germany (*J. Exp. Med.*, 1999)
Mature DC pulsed with Mage-3A1 and a recall antigen (TT or tuberculin) in 11 **stage IV melanoma patients**

Expansion Mage 3-A1 specific CTL in 8/11 patients which declined after the i.v injection.
Regression of individual metastases in 6/11 patients

DC-based Vaccine



Mature or immature DC

- Immature and mature monocytes derived DCs were separately pulsed with a peptide derived from tyrosinase, MelanA/MART-1 or MAGE-1 and a recall antigen. Both DC populations were injected every 2 weeks in different lymph nodes.

- Mature DCs induced increased recall antigen-specific $CD4^+$ T-cell responses in 7/8 patients, while immature DCs did so in only 3/8 (Jonuleit H et al. Int J Cancer 2001).

- Expansion of peptide-specific IFN-gamma-producing $CD8^+$ T cells was observed in 5/7 patients vaccinated with mature DCs but in only 1/7 using immature DCs (Jonuleit H et al. Int J Cancer 2001)

Mature or Immature DCs

The capacity of BmDC to induce an anti-tumour immune response *in vivo* correlated to their degree of maturation (Labeur MS et al. J Immunol 1999)

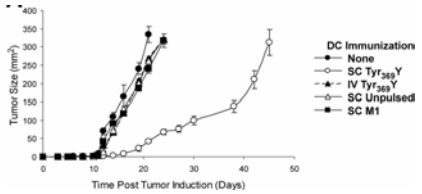
Impaired anti-tumour responses in the absence of CD40/CD154 interactions are the result of a lesion in APC function, namely IL-12 production (Mackey MF et al. J Immunol 1998)

Route of immunization

DC injected s.c or intradermally migrate to the lymph node. But only 0.4 to 1% of DCs injected s.c reached the draining lymph node (Kupiec-Weglinski JW et al. J Exp Med 1988; Lappin MB Immunology 1999)

DC administered i.v preferentially migrate to the spleen (< 15% of the number injected) and the lung with no migration or only trace to regional lymph node (Morse MA et al Cancer Res 1999; Barratt-Boyes SM et al. J Immunol 1997)

In mouse, superiority of s.c injection of DCs over i.v injection in the induction of CTL and anti-tumour immunity against subcutaneously growing tumours (Okada N et al. Br J Cancer 2001; Eggert A et al. Cancer Res 1999; Serody JS et al. J Immunol 2000)



s.c but not i.v immunization with peptide pulsed DCs controls s.c melanoma outgrowth.

Mullins DW. J Exp Med 2003

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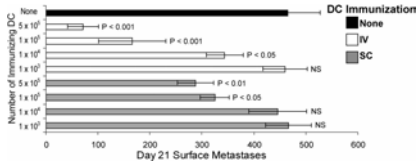
- I.V peptide pulsed activated DC immunization induces memory T cells in the spleen, control metastatic-like lung tumours but not subcutaneous growing tumours (Mullins DW et al. J Exp Med 2003).



Metastatic like lung lesions are controlled by i.v immunization and partially by s.c immunization with peptide pulsed DCs

Mullins DW J Exp Med 2003

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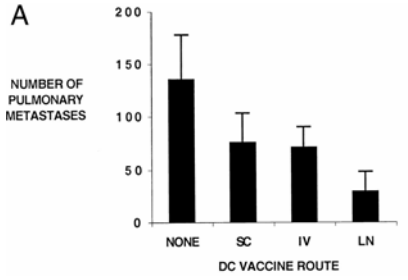
- In human, i.v purified DC administration was associated with a significantly higher frequency and titre of Ag-specific Abs (Fong L. J Immunol 2001)

Pulse of DC with antigen coupled to a carrier (KLH) allows an increase in the frequency of antibodies production and IgG isotype (Timmerman JM Blood 2002).

Intranodal immunization

- Bone marrow-derived, tumour lysate-pulsed DCs administered intranodally generated more potent protective anti-tumour immunity (pulmonary metastases) than s.c or i.v DC immunization. This was associated with greater antigen-specific T-lymphocytes expansion (Lambert LA. Cancer Res 2001).



Intranodal immunization with TS/A tumour lysate-pulsed DCs is more effective than s.c or i.v immunization

Lambert LA. Cancer Res 2001

Intranodal immunization

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- In human, patients were randomly assigned to an intravenous, intranodal or intradermal route of peptide pulsed activated DC immunization.

All routes of immunization induced comparable increases in tetramer-staining $CD8^+$ T cells. However, the intranodal route induced significantly higher rates for de novo development of $CD8^+$ T cells that respond by cytokine secretion to peptide-pulsed targets (Bedrosian I et al. J Clin Oncol 2003)

Route of Administration and Dose	Tetramer Positivity: No. of Patients With Response	Tetramer Positivity: No. of Patients Treated	Peptide Reactivity: No. of Patients With Response	Peptide Reactivity: No. of Patients Treated	DTH Positivity: No. of Patients With Response	DTH Positivity: No. of Patients Treated
IV						
5 million dendritic cells	3	4	0	4	0	4
50 million dendritic cells	2	3	0	2	1	3
Total No. of patients	5		0		1	
Total No. of patients		7		6		7
Patients with response, %		71.4		0		14.3
IN						
5 million dendritic cells	3	5	4	5	5	5
50 million dendritic cells	1	2	2	2	2	3
Total No. of patients	4		6		7	
Total No. of patients		7		7		8
Patients with response, %		57.1		85.7		87.5
ID						
5 million dendritic cells	2	3	2	3	2	3
50 million dendritic cells	2	3	0	3	0	3
Total No. of patients	4		2		2	
Total No. of patients		6		6	6	
Patients with response, %		66.7		33.3	33.3	
P, by Fisher's exact test applied to totals		.99		.005	.01	

Abbreviations: DTH, delayed-type hypersensitivity; IV, intravenous; IN, intranodal; ID, intradermal.

Tumour-specific peptide reactivity is enhanced after intranodal immunization

Bedrosian I. J Clin Oncol 2003

Route of immunization and polarization

- KLH-pulsed bone marrow-derived DCs were shown to stimulate a TH-1 cytokine response in the draining lymph node when administered s.c and a TH2 cytokine response when administered i.v (Morikawa Y et al. Immunology 1995)

- Bone marrow-derived, tumour lysate-pulsed DCs administered intranodally generated more potent antigen-specific TH1-type response than when injected s.c or i.v (Lambert LA. Cancer Res 2001)

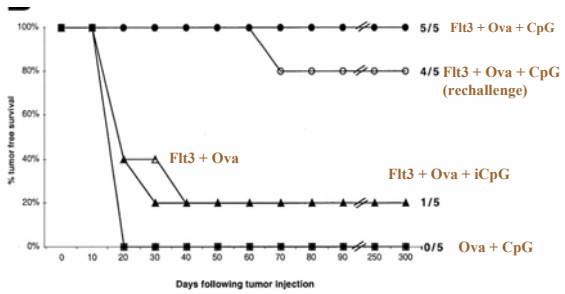
- In human i.d and intralymphatic administrations of purified DC induce TH1 immunity with greater frequency than i.v administration (Fong L et al. J Immunol 2001)

Generation *in vivo* of mature DC

Flt3 + CpG

Administration of a bone marrow growth factor Flt3 has been shown to expand in vivo the numbers of DCs in lymph nodes, spleen and other tissues (Maraskovsky et al. J Exp Med 1996)
However, a significant proportion of the FL-mobilized DCs are immature and not efficient in inducing Ag-specific T-cell responses.

In mice pretreated with FL, the simultaneous delivery of a tumor antigen and CpG induce potent anti-tumour immunity (Merad M et al. Blood 2002).



Antigen loading and CpG activation of FL-mobilized DCs induce tumour protection

Merad M Blood 2002

Correlation between immune response and clinical response ?

Some studies did not demonstrate any correlation between the induction of immune response and anti-tumour activity.

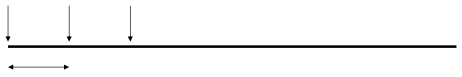
Immunisation of 11 melanoma patients with gp100 modified peptide
$gp100_{209\text{-}2M}$ + IFA: 10 T cell response (CD8) (90%)
Clinical response : No

Immunisation of 19 melanoma patients with gp100 modified peptide
$gp100_{209\text{-}2M}$ + IFA + IL-2: 3 T cell response (CD8) (15%)
Clinical response : 42%

Rosenberg Nature Med 1998.

Vaccination of metastatic melanoma patients with Mage 3-A1 peptide

Peptide Mage 3

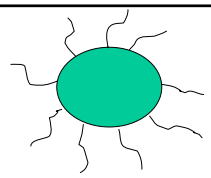


39 patients included.
No toxicity
25 patients received the complete treatment :
4 PR and 3 CR

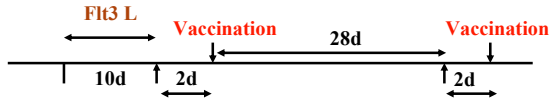
No detection of CTL directed against Mage 3

Marchand et al 1999.

… Whereas other groups reported a correlation between the induction of specific T cells and clinical effects



Pulsed DC with modified peptide
CEA 605-613$^{Asp\text{-}Asn\ (610)}$

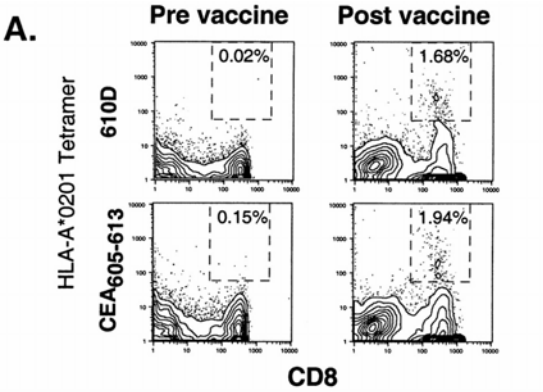


- Cytapheresis
- DC purification
(Density gradient)

- Cytapheresis
- DC purification
(Density gradient)

Phase I: 12 patients
(Colon adenocarcinomas and non small lung carcinomas)

A.

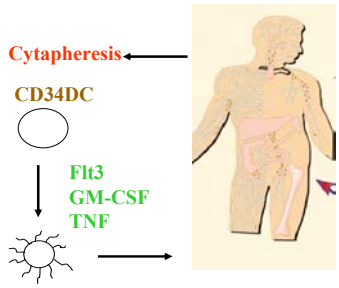


Identification and characterization of antigen-specific CD8 T cells with MHC/peptide tetramers

Correlation between immune and clinical responses* .

Patient	Clinical Response	Tetramer$^+$ Prevaccine%	Tetramer$^+$ Postvaccine%
1	PD	0.08	0.25
2	PD	0.03	0.08
3	SD	0.15	1.11
4	PD	0.18	0.04
5	CR-10Mo	0.4	1.03
6	PD	0.10	0.31
7	PD	0.26	0.49
8	SD	0.43	1.05
9	PD	0.16	0.07
10	PD	0.24	0.5
11	CR-10Mo	0.28	1.03
12	MR	0.12	1.68

* P = 0.002 Fong L. PNAS 2001



18 metastatic melanoma patients

Pulse: Tumour peptides → Melan A, tyrosinase,Mage 3, gp100.
Control peptide → Flu-MP
Control protein → KLH

Banchereau Cancer Res 2001

RESULTATS

Cellular response against control antigens:
(CD4 anti-KLH and/or CTL anti Flu-MP): 16/18 patients.

↗ CTL against ≥ 1 tumor peptide derived from melanoma Ag: 16/18

T-CD8 response	> 2 Mel peptide	< 2 Mel peptide
Tumour progression	1/10	6/7
Regression of at least 1 metastasis	7/10	0/7

P =0.015

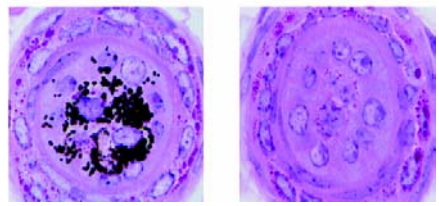
Vitiligo: 2/17 Patients
(responder patients)

AUTO-IMMUNITY AND CANCER

- Anti-tumour CD8-CTL in cancer patients mostly recognize melanoma melanocyte differentiation antigens (Melan A, gp100, TRP1 et 2, Tyrosinase…)

AUTO-IMMUNITY AND CANCER

rVVLacZ rVVmTRP-1



Immunization with rVVTRP-1 induces destruction of cutaneous melanocytes.

Overwijk W et al. PNAS 1999

AUTO-IMMUNITY AND CANCER

- Anti-tumour CD8-CTL in cancer patients mostly recognize melanoma melanocyte differentiation antigens (Melan A, gp100, TRP1 et 2, Tyrosinase…)

- Cancer patients responding to IL-2 therapy more often develop vitiligo and auto-immune thyroiditis than non responders. (Rosenberg SA 1996).

- Autoimmune paraneoplastic syndrome is often associated with anti-tumour clinical response. (Darnell RB Lancet 1993)

POTENTIAL MECHANISMS LEADING TO TUMOR ESCAPE FROM IMMUNE RECOGNITION

POTENTIAL MECHANISMS LEADING TO TUMOUR ESCAPE FROM IMMUNE RECOGNITION

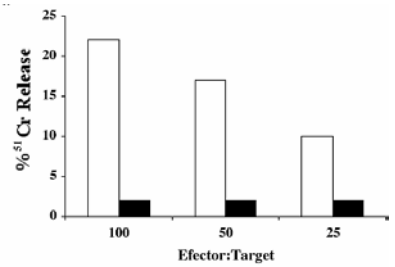
- Antigen loss or heterogeneity of antigen expression in tumours

- Partial or global down-regulation of MHC class I molecules on tumours (β2 microglobulin loss, peptide transporter defect)

- Immunosuppressive factors (IL-10, TGFβ, PGE2…) in the tumour microenvironment.

- High expression of molecules associated with tumour resistance to immune attack

- Fas ligand expression on tumour cells

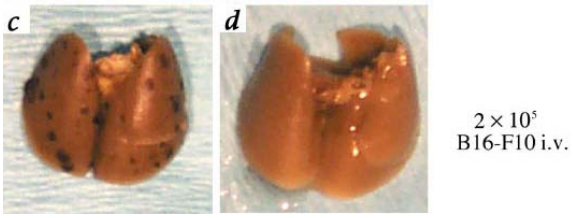


In vivo generation of tumour-specific $CD8^+$ CTLs in the absence of TGFβ- signaling in T cells.

Spleen cells were isolated from transgene-positive (□) or transgene-negative (■) littermate mice challenged with 10^6 live EL-4 cells 10 days prior and were evaluated for their lytic activity by the 51Cr release assay against EL-4 targets.

Gorelik L Nature Med 2001

Wild Type Mice **DN TGFRII Mice**



Blockade of TGF-signaling in T cells renders mice resistant to tumour challenge.

Gorelik L. Nature Med 2001

Molecular or functional defects of T lymphocytes infiltrating tumours.

- Defect in the expression of the ζ chain of -CD3

- Defect in the expression of transcription factors belonging to NF-Kappa B family
  - . p65-Rel A
  - . c-Rel
  - . p50
- Defect in the expression of Jak 3

- Low perforine expression within T cells

- Expression of KIR by TIL

- Bias in TH2 polarization of TIL

Presence of «suppressive» T lymphocytes

- During cancer progression, increase in the frequency of regulatory T lymphocytes ($CD4^+CD25^+$, or Tr1 producing IL-10 or TGFβ or NKT secreting IL-13) able to inhibit the activity of specific anti-tumour lymphocytes.

- In murine models, depletion of $CD4^+CD25^+$ T lymphocytes increases the induction of immune response and is associated with tumour regression.

Cancer Vaccine Indications

- Residual Disease

- Adjuvant
  (After surgery if presence of risk factors)

- Genetic predisposition to cancers

PERSPECTIVES

- **Molecular identification of tumour antigen associated with the rejection of tumours.**

- **Better definition of the phenotype of CD8 T cells to be induced after vaccination: avidity Tc1-Tc2, duration of the persistence of the T cell response**

- **Better understanding of the factors responsible (chemokines, adhesion molecules) of the tumour homing of T lymphocytes.**

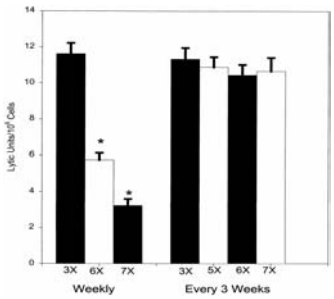
Table 1 Percentage of PR1-specific CTL from PBMC of CML patients

Treatment Group	UPN	Stage[3]	Time from Diagnosis[5]	Treatment Duration[5]	%Ph[3]	Response Type*	%PR1-CTL[1]
Interferon	1	CP	15	15	54	MR	1.06
	2	CP	20	18	40	MR	1.02
	3	CP	5	3	75	MR	.51
	4	CP	40	11	38	MR	1.52
	5	CP	18	18	0	CR	.42
	6	CP	10	10	28	PR	.66
	7	CP	9	9	25	PR	.43
	8	CP	10	10	10	PR	.32
	9	CP	14	14	0	CR	1.24
	10	CP	16	16	10	PR	.29
	11	CP	12	12	4	PR	.43
$P = 0.0002$							
	12	CP	42	40	85	MR	< 0.01
	13	CP	12	12	100	None	< 0.01
	14	BC	4	4	100	None	< 0.01
	15	BC	2	2	100	None	< 0.01
	16	CP	5	5	95	None	< 0.01
	17	CP	20	16	100	None	< 0.01
	18	CP	30	24	100	None	< 0.01
	19	CP	15	13	100	None	< 0.01

Molldrem Nature Med 2000

2nd PSU Workshop on Tumour Immunology and Immunotherapy, December 15-20, 2003. Prince of Songkla University, Université Pierre et Marie Curie and Institut Pasteur

Schedule of DC administration



Weekly administration of peptide-pulsed DCs led to diminishing CTL activity after 6 wk of treatment. This was not found in animals injected with DCs every 3 wk for six treatments

Serody JS J Immunol 2000

2nd PSU Workshop on Tumour Immunology and Immunotherapy, December 15-20, 2003. Prince of Songkla University, Université Pierre et Marie Curie and Institut Pasteur