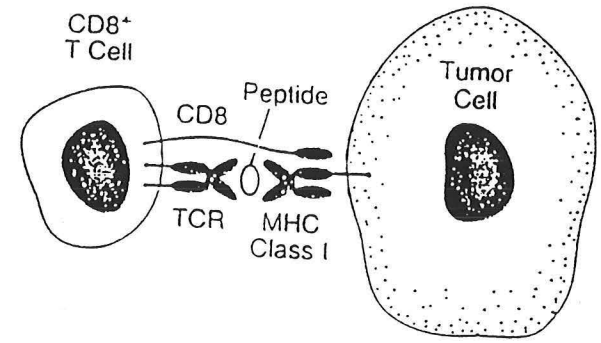
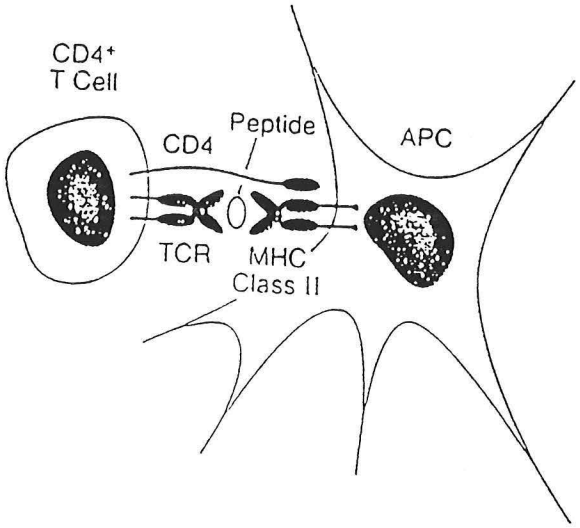
Biologic Therapy of Melanoma





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"This is to acknowledge that Barry Levinson, M.D., has disclosed no financial interests or other relationships with commercial concerns related directly or indirectly to this program."

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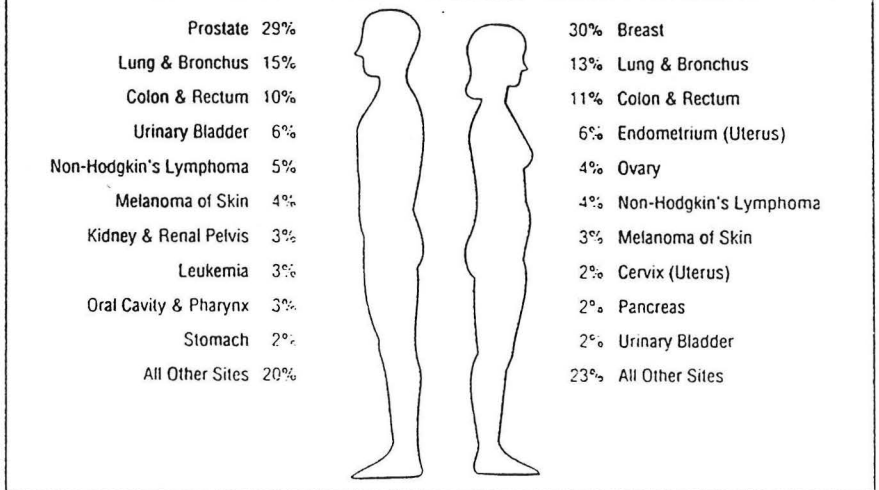
Interests include clinical research in a variety of solid tumors including melanoma, renal cell carcinoma, and gastrointestinal malignancies.

Introduction

Melanoma is rapidly increasing in incidence. There will be an estimated 41,600 new cases and 7,300 deaths due to melanoma in 1998.[1] Over 80 percent of patients are diagnosed with localized disease, and usually have a good prognosis. Unfortunately, the five year survival for those with lymph node involvement is approximately 40%, and less than 10% for patients with metastatic disease. Surgery is of greatest benefit for patients with localized or local regional disease. There is also a role for resection of selected metastatic lesions. Systemic treatment with chemotherapy has not improved overall survival in patients with metastatic disease. Dacarbazine (DTIC) the chemotherapy drug which has been used as a standard of treatment for metastatic melanoma has a response rate of only 15%. Combinations with other drugs such as cisplatin, BCNU, and vinblastine has improved response rates even to the 30% range but the overall impact on survival is unchanged.[2]

CANCER STATISTICS, 1998

Figure 1
Estimated New Cancer Cases*
10 Leading Sites by Sex, United States, 1998



*Excludes basal and squamous cell skin cancer and carcinoma in situ except bladder.

Reference 1

Several observations were made which suggested that melanoma is capable of eliciting an immune response:

- the well documented finding of spontaneous remissions
- the presence of "halos" around nevi
- the finding of lymphocyte infiltration in areas of tumor regression

These findings, when coupled with the overall poor outcome of conventional chemotherapy in the treatment of advanced disease, led investigators to explore the potential effects of biologic therapy in the treatment of melanoma.
Biologic therapy differs from the traditional modes of cancer therapy; surgery, radiation therapy or chemotherapy by stimulating natural host defense mechanisms, predominantly the immune system, to mediate regression of disease.[3] Two important advances have been made which have allowed biologic therapy to grow as a field of investigation. Greater understanding of the amazingly complex role of the body's immune system as it relates to tumors and their antigenicity; and the availability of biologic agents, especially cytokines, which signal cells active in the immune response.

American Joint Committee on Cancer Staging of Melanoma

Primary Tumor (pT)		
pTX		Primary tumor cannot be assessed
pT0		No evidence of primary tumor
pTis		Melanoma in situ (atypical melanocytic hyperplasia, severe melanocytic dysplasia), not an invasive lesion (Clark's Level I)
pT1		Tumor 0.75 mm or less in thickness and invading the papillary dermal (Clark's Level II)
pT2		Tumor more than 0.75 mm but not more than 1.5 mm in thickness and/or invades to the papillary-reticular dermal
pT3		Tumor more than 1.5 mm but not more than 4 mm in thickness and/or invades the reticular dermis (Clark's Level IV)
	pT3a	Tumor more than 1.5 mm but not more than 3 mm in thickness
	pT3b	Tumor more than 3 mm but not more than 4 mm in thickness
pT4		Tumor more than 4 mm in thickness and/or invades the subcutaneous tissue (Clark's Level V) and/or satellite(s) within 2 cm of the primary tumor
	pT4a	Tumor more than 4 mm in thickness and/or invades the subcutaneous tissue
	pT4b	Satellite(s) within 2 cm of the primary tumor

Regional Lymph Nodes (N)		
NX		Regional lymph nodes cannot be assessed
N0		No regional lymph node metastasis
N1		Metastasis 3 cm or less in dimension in any regional lymph node(s)
N2		Metastasis more than 3 cm in greatest dimension in any regional lymph node(s) and/or in-transit metastasis
	N2a	Metastasis more than 3 cm in greatest dimension in any regional lymph nodes
	N2b	In-transit metastasis
	N2c	Both (N2a and N2b)

Distance Metastasis (M)		
MX		Presence of distance metastasis cannot be assessed
M0		No distance metastasis
M1		Distant Metastasis
	M1a	Metastasis in skin or subcutaneous tissue or lymph node(s) beyond the regional lymph nodes
	M1b	Visceral metastasis

Note: In-transit metastasis involves skin or subcutaneous tissue more than 2 cm from the primary tumor not beyond the regional lymph nodes.

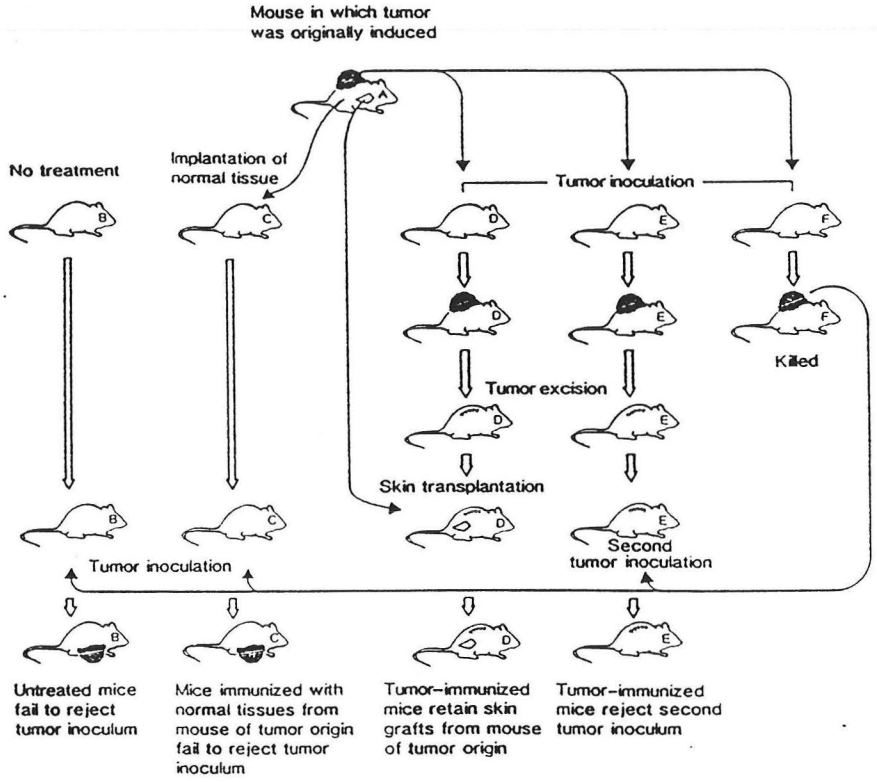
STAGE GROUPING

Stage 0	pTis	N0	M0
Stage I	pT1	N0	M0
	pT2	N0	M0
State II	pT3	N0	M0
Stage III	pT4	N0	M0
	Any pT	N1	M0
	Any pT	N2	M0
Stage IV	Any pT	Any N	M1

From the Manual of Staging of Cancer (ed. 5). Philadelphia, PA, Lippincott, 1998, pp 163-167.

Elements of Tumor Immunology

Understanding of tumor immunology was initially elaborated through murine experiments.[4] Chemically induced sarcomas in mice were surgically removed and each mouse was subsequently immunized with x-irradiated cells from the sarcoma. Subsequently, the transplant of living sarcoma cells was rejected in an antigen specific way, each sarcoma expressing its own antigen.[5] Transplant studies of mouse sarcomas indicated that the ability of an immunized mouse to reject a tumor was adoptively transferred by lymphocytes and not by serum. Further studies demonstrated that the lymphocytes in the immune response were T lymphocytes. The immune response to viral induced tumors was also mediated through lymphocytes; antibodies did not appear to play an appreciable role. These studies demonstrated that tumor rejection is predominantly mediated through cellular immunity and not humoral immunity. In contrast to chemically and viral induced tumors, spontaneous tumors were not associated with tumor rejection antigens.[6]



Fundamental Immunology, 3rd Ed. WE Paul. Raven Press, New York 1993.

T Lymphocyte Response to Antigen

Cellular immune response requires the direct participation of effector cells such as T lymphocytes. Functions of both T helper ($CD4^+$) and cytotoxic T lymphocytes ($CD8^+$) are activated by the binding of specific antigens composed of protein. Antigens are presented on the target cell in association with major histocompatibility complex (MHC), to the T-cell receptor (TCR) molecules which are receptors for peptide antigens. Thus T-cell action is considered antigen specific and MHC restricted. Antigen is denatured, cleared within the cell and transported into specific subcellular compartments where it is bound by MHC molecules. After a complex of antigen and MHC completes its journey to the cell surface, it is potentially recognized by a T-cell.[7]

T cell receptors (TCR) recognize peptide fragments of antigens called epitopes. These are non-covalently complexed with MHC molecules. Two paired proteins form a transmembrane non-secreted heterodimer unique for each clone of T cells which determines the antigen specificity of the TCR. There are αβ heterodimers and γδ. αβγδ encode for the TCR function via rearrangement from the germ cell line configuration. Mature T-cells express either a αβ or γδ heterodimer. Nearly all T-cells, characterized to recognize specific antigen in an MHC restricted fashion, express αβ receptors. Antigen specificity of γδ TCR is largely MHC - unrestricted.[8] MHC class I molecules are recognized by $CD8^+$ cells and MHC class II molecules are recognized by $CD4^+$ cells.

Upon activation, helper T-cells secrete cytokines (IL2, Interferon α, GM-CSF, TNFα) which promote the recruitment and activation of other cells, such as macrophages, to execute their effector functions. In contrast, cytotoxic T lymphocytes (CTL) result in the direct killing of target cells. This is accomplished through the action of soluble cytolytic mediators (perforin and granzyme) stored in cytoplasmic granules in the T cell or through a killer lymphocyte surface molecule (Fas ligand)[9,10]. NK cells do not express surface molecules for T or B lymphocytes and are capable of lysing a variety of target cells without antigen stimulation and in a non-MHC restricted fashion.

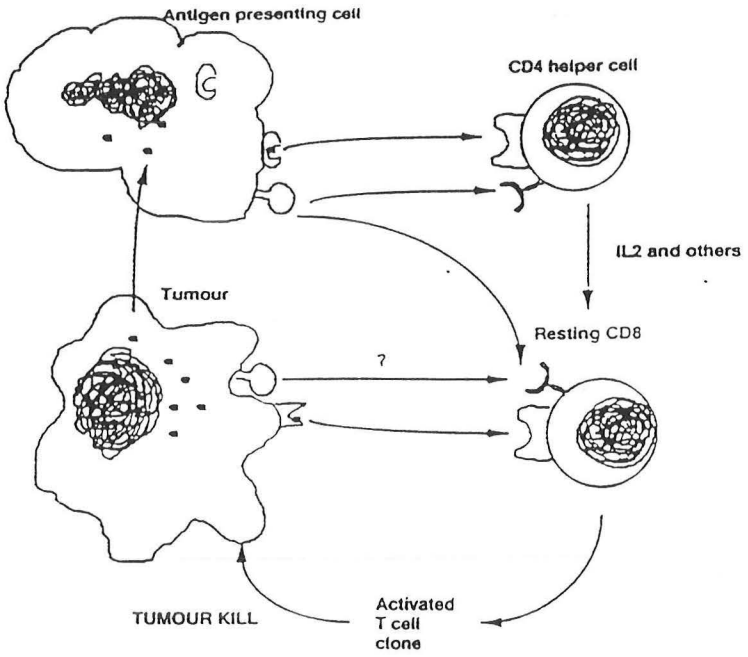


Fig. 1 Cell mediated tumour immunity. Tumour antigen, for instance MAGE-1, is released and is taken up by the antigen presenting cell. Antigen is processed and presented to T-helper and resting cytotoxic T-cells, a stimulus that is relatively specific for antigen and T-cell, and requires the co-stimulatory cell surface molecule B7 to avoid anergy. In the presence of IL-2 and other lymphokines, probably IL-4 and IL-12m cytotoxic T cells are activated and recognise antigen presented by the tumour cell by MHCII. Points of intervention includ enhancement of MHC, B7 or antigen, stimulation with cytokines or the piecemeal replacement of antigen presenting cells or lymphocytes.

Bridgewater JA, Gore ME: Biological response Modifiers in melanoma. Br Med Bull 51: 656-677, 1995.

Class I and Class II MHC molecules are integral membrane glycoproteins. MHC class I molecules are heterodimers composed of a 45,000 Mr glycoprotein - heavy chain non-covalently associated with the 11,600 Mr B2 microglobulin (light chain). MHC Class I heavy chains are integral membrane proteins consisting of 2 amino-terminal extracellular polymorphic domains (α1 and α2) followed by a constant extracellular domain (α3), a hydrophobic trans-membrane segment that anchors the molecule to the plasma membrane, and a short carboxyl terminal cytoplasmic domain. Class I molecules are found on most tumor cells and somatic cells. Class II molecules are found on only a subset of tumor cells and also B cells, macrophages, Langerhans cells and follicular dendritic cells, so-called professional antigen presenting cells (APC).[11] These APC also express co-stimulatory molecules on their surface (e.g. B7 family of ligands). The co-stimulatory molecules (signal 2) are crucial to the effectiveness of the immune activation associated with the presentation of antigenic peptides bound to MHC class I molecules (signal 1). Immune activation depends on the recruitment of APC to the site of tissue damage where these cells can phagocytize and process potentially antigenic components to be presented to TCR of cognate T cells. Failure to express the proper MHC molecules for antigen presentation or proper co-stimulatory molecules can prevent an efficient antitumor immune response.[12]

Escape From Immunologic Control

Several mechanisms provide escape from immunologic control. Co-stimulating signal results from interaction between an adhesion molecule expressed on the T cells and its ligand expressed on the antigen presenting macrophage. One such molecule is CD28/CTLA-4 receptor whose ligand is B7. This ligand was first detected on activated B cells and is present on other antigen presenting cells as well. Abrogation of the interaction between CD28/CTLA-4 and B7 inhibits the development of an effective tumor response.[13] In some cases this inhibition is sufficent to induce long-term immunologic nonreactivity.

The release of antigen from tumor cells either alone or in an immune complex can block an immune response to itself. This appears to play a key role in facilitating the escape of antigenic tumors from immunologic control. This mechanism has been attributed to suppressor T cells.

Circulating tumor cell antigens may be taken up by APC which lack B7 and there are degraded into peptides and presented to T cells, where if there is no costimulation, become anergic and take on a suppressor function.[14]

Tumor cells may also dampen the immune response by secreting inhibitory cytokines such as IL10; TGFβ 1, 2 and 3 and IL-1 receptor antagonists.

Melanoma Specific T-cell Mediated Immune Response

Four basic approaches have been used to detect and define tumor antigens which can elicit an immune response:[15]

- analysis of sera of cancer patients and normal individuals
- development of monclonal antibodies from lymphocytes of cancer patients
- analysis of cytotoxic T lymphocytes from cancer patients
- analysis of specificity of immune responses in patients receiving whole cell or purified vaccines

Using T cell cloning technology, several laboratories in the 1980's were able to isolate autologous melanoma specific CTL clones from patients with melanoma. Cells were isolated

from peripheral blood, lymph nodes and tumor tissue. These were αβ T cells which required TCR for proper function and selectively recognized an antigen in association with the self MHC molecule. Intially it appeared as though these CTL's recognized unique antigens associated only with autologous melanoma cells, but it was discovered that some of these CTL clones also recognized certain allogeneic melanoma cells. These types of CTL lines and the allogeneic melanoma cells shared an MHC class 1 molecule between them.

In most of these cases of shared melanoma antigen recognition, the MHC recruiting element was HLA-A2, a haploptype expressed by approximately 50% of the caucasian population. The tyrosinase gene has been identified to code for a number of peptides that are recognized by CTL in association with the HLA-A2 molecule. There is a considerable degree of diversity in the TCR usage by CTL in recognition of a peptide on a specific MHC molecule.

Human tumor antigens recognized by T cells.

Tumor antigens	Restriction element	Peptide epitope
Melanoma antigens		
Melanocyte lineage proteins		
gp100	HLA-A2	KTWGQYWQV
	HLA-A2	ITDQVPFSV
	HLA-A2	YLEPGPVTA
	HLA-A2	LLDGTATLRL
	HLA-A2	VLYRYGSFSV
MART-1/MelanA	HLA-A2	AAGIGILTV
	HLA-A2	ILTVILGVL
TRP-1 (gp75)	HLA-A31	MSLQRQFLR
Tyrosinase	HLA-A2	MLLAVLYCL
	HLA-A2	YMNGTMSQV
	HLA-B44	SEIWRDIDF
	HLA-A24	AFLPWHRLF
	HLA-DR4	QNILLSNAPLGPQFP
	HLA-DR4	SYLQDSDPDSFQD
Tumor-specific, widely shared antigens		
MAGE-1	HLA-A1	EADPTGHSY
	HLA-Cw16	SAYGEPRKL
MAGE-3	HLA-A1	EVDPIGHLY
	HLA-A2	FLWGPRALV
BAGE	HLA-Cw16	AARAVFLAL
GAGE-1, -2	HLA-Cw6	YRPRPRRY
N-acetylglucosaminyltransferase-V	HLA-A2	VLPDVFIRC
p15	HLA-A24	AYGLDFYIL
Tumor-specific, mutated antigens		
β-catenin	HLA-A24	SYLDSGIHF*
MUM-1	HLA-B44	EEKLIVVLF*
CDK4	HLA-A2	ACDPHSGHFV*
Nonmelanoma antigens		
HER-2/neu	HLA-A2	IISAVVGIL
(Breast and ovarian carcinoma)	HLA-A2	KIFGSLAFL
Human papillomavirus-E6, E7	HLA-A2	YMLDLQPETT
(Cervical carcinoma)		
MUC-1	Non-MHC-restricted	PDTRPAPGSTAPPAHGVTSA
(Breast, ovarian and pancreatic carcinoma)		

*Mutations are noted in bold.

Robbins and Kawakami: Human tumor antigens. Curr Opin Immunol 8: 628-636, 1996.

$CD4^+$ T cells can also participate in the immune response to melanoma. A significant number of melanomas do express MHC Class II products and can present peptides on their MHC Class II molecules. Professional APC can also present peptides from melanoma antigens and induce a $CD4^+$ T-cell response. Using the melanoma antigens against which CTL clones were identified, the structure of the genes which coded for these antigens were deciphered. Using techniques in which complementary DNA derived from an antigen positive parent line was transfected into an antigen negative variant, selected for antigenic loss with the MHC gene expression preserved, Van der Bruggen et al[16] were able to clone the gene that expressed the MZ2E CTL determined melanoma antigen. The gene was termed MAGE-1. Three additional members of the MAGE family have been characterized (2,3,4). MHC Class I molecule HLA-A1 is the restriction element for MAGE-1. This antigen is not found in normal tissue except the testis. Other members of this family of proteins include BAGE, GAGE, Mel40 and ESO-1 and are located on the X chromosome. They are often expressed in later stages of tumor development.

Differentation Antigens

The differentiation antigen tyrosinase (required for melanin synthesis) and other peptides derived from it, are HLA-A2 restricted antigens. They include MART-1 (melanoma antigen recognized by T-cells) glycoprotein 100 (gp 100) and Melan-A. As opposed to MAGE-1, differentiation antigens are expressed in normal melanocytes and pigmented retinal cells in addition to melanoma cells. Since these antigens are present in non-mutated cells, it may be the overexpression or higher concentration of these peptides which may be perceived as abnormal. Melanocytes are distinctive for their ability to synthesize melanoma pigment in the melanosome (the site for expression of several melanocyte specific gene products).

Differentiation Pathway for Melanocytes

- Expression of cell surface and intracellualar differentiation antigen
- Presence or absence of melanin
- Stage of melanosome development
- Expression of tyrosinase, major catalytic enzyme in the synthesis of melanin
- Cell morphology

Melanoma cells and melanocytes at a late stage of differentiation are characterized by: expression of late differentiation antigens, melanin, high tyrosinase activity and dendritic cell morphology.

These antigens have become a focus of investigation, both to enhance the immune response and to gauge the effectiveness of therapies. Prior to the discovery of these melanoma-associated and differentiation antigens the focus of immunologic therapies was more generalized.

Active Non-Specific Immunomodulation

A century ago, the observation that erysipelas infections appeared to be associated with some regressions of tumors led Coley[17] to inject bacterial toxins directly into tumors. He reported some interesting responses but the practice was ultimately abandoned. Nonetheless, this is the first modern report of biologic therapy in the treatment of malignancy. Subsequently in the 1960's BCG was used as a non-specific immunostimulant and actual tumor regression was noted when it was injected directly into cutaneous melanoma lesions.[18,19] Overall BCG was ineffective as systemic therapy[20] but it still is administered with some tumor vaccines as an adjuvant to

enhance immunogenicity. Other non-specific immune therapies such as Corynebacterium parvum and levamisole were tried in melanoma patients without significant response. For the past two decades, potent and effective immunostimulants have been used extensively as non-specific immune modulators in the therapy of melanoma, namely Interferon and Interleukin 2.

Interferon

Interferon (IFN) can have potent immunostimulatory effects. IFN is able to stimulate NK cells and cytotoxic lymphocytes and can upregulate cell surface antigens or tumor in vitro, in particular MHC I and II molecules responsible for the presentation of tumor antigens on the cell surface. Interferon has anti-proliferative activity in vitro, as well, as anti-angiogenic effects.

Type I interferons include interferon alpha (IFN-α) and interferon beta (IFN-β). Type II interferons include interferon gamma (IFN-γ). Interferon-α is produced by lymphocytes and macrophages, Interferon-β is produced by fibroblasts and epithelial cells and Interferon γ is produced by T lymphocytes and NK cells. IFN-α and IFN-β are closely linked in structure and both appear to have greater antiviral activity and less immunomodulatory activity as compared to IFN-γ. Nonetheless most studies evaluating the role of IFN therapy in melanoma, either in metastatic disease or as post-operative adjuvant therapy have used IFN-α (IFN-α2a or IFN-α2b).[21]

Despite the favorable immunologic effects of IFN-γ, it has proven ineffective as adjuvant therapy in a Southwest Oncology group randomized trial. There was a suggestion that the IFN-γ arm fared worse, prompting early disclosure of results.[22]

Interferon α has been used in a variety of modes of administration (intravenous, intramuscular, subcutanous), a wide variety of doses (2 million units/m^2 to 20 million units/m^2) and an equally broad range of schedules (intermittent or continuous) from weeks to years. The overall response rate in metastatic disease is 16% with 5% of patients demonstrating a prolonged duration of remission. Higher doses and prolonged schedules appear to be associated with improved response rates.[23]

IFN-α has also been used widely in studies as an adjuvant therapy and it is in this setting that interferon has had an impact on clinical practice. One report from Kokoschka[24] used a low dose subcutaneous schedule for 20 months. There were 65 patients (53 had stage I disease) in the treatment arm compared to 115 concurrent controls. (82 had clinical stage I disease). There was no statistical improvement in overall or disease free survival over 5 years but there was a lower rate of relapse while on the interferon therapy.

Creagan at the North Central Cancer Treatment Group[25] enrolled 262 patients with stage I or stage II disease treated with 20 mu/m^2 of IFN-α2a intramuscularly three times a week for twelve weeks. There was no improvement in overall or disease free survival.

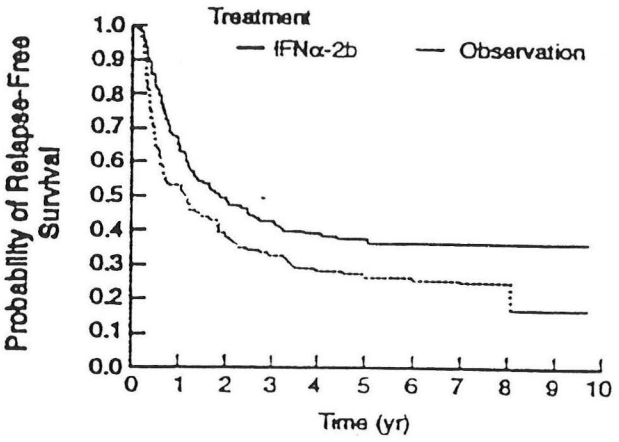
Cascinelli at the World Health Organization (WHO)[26] evaluated 444 patients with pathologic stage III disease, with positive lymph nodes. Patients were treated with 3 million units subcutaneously three times a week for 3 years. There was no overall improvement or disease free survival.

Kirkwood et al.[27] reported on an Eastern Cooperative Oncology Group (ECOG), trial which randomized 287 patients with positive lymph nodes, primary lesions › 4mm in depth, or recurrent lymph node disease. Half the patients were randomized to a high dose interferon with a 4 week induction period using IFNα2b 20mu/m^2 IV day 1-5/week followed by a maintenance period of 48 weeks with a dose of 10 MU/m^2 subcutaneously three times a week. As

expected, the toxicity of high dose interferon was great. Most common adverse effects were fatigue, myelosuppression, fever, myalgias, anorexia, nausea, derangement of liver function tests (the only two treatment related deaths were from liver toxicity) and headache. Twenty four percent of patients discontinued treatment because of adverse effects, 65% of patients had dose modification secondary to toxicity.

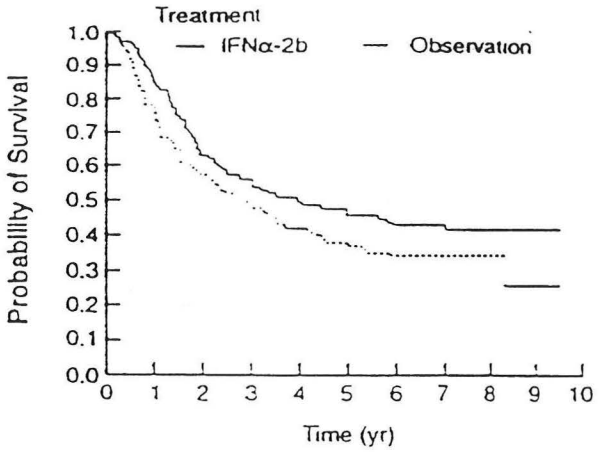
There was a statistically significant improvement in relapse free survival: (37%) for the treatment arm and (24%) for the control arm. Median relapse free survival was increased by 9 months. There was a statistically significant increase in overall 5 year survival. 46% for the interferon arm vs. 37% for observation with an increased median overall survival by 1 year (3.82 years vs. 2.78 years).

RELAPSE-FREE SURVIVAL



	No. Patients	No. Relapsed	Median (yr)	P Value	5-Year RFS
IFNα-2b	143	88	1.72	<.01	37%
OBS	137	101	.96		26%

OVERALL SURVIVAL



	No. Patients	No. Dead	Median (yr)	P Value	5-Year Survival
IFNα-2b	143	76	3.82	.047	46%
OBS	137	85	2.78		37%

This was the first study to demonstrate a clear benefit to the adjuvant use of interferon. As a consequences of this study high dose interferon has become a standard of care for post operative stage III patients. This is a highly toxic regimen and not easily tolerated by many patients. The results of other randomized trials, an Intergroup trial and another from the EORTC are pending.

Interleukin- 2 and Adoptive Immunotherapy

Interleukin-2 (IL-2) was originally called T-cell growth factor. IL-2 is a glycoprotein which is produced by antigen or mitogen activated T-cells and promotes proliferation of cytotoxic T-cells and natural killer cells.

Initially studies utilizing IL-2 as an antitumor therapy were carried out in conjunction with adoptive immunotherapy, that is; the transfer to the tumor bearing host of immunologically

reactive cells with antitumor reactivity that can mediate antitumor effect either directly or indirectly.
The first such cells utilized by Rosenberg at the NCI, were lymphokine activated killer (LAK) cells.[28] These are non-B, non-T cells capable of recognizing cancer cells in a non-MHC restricted fashion. When incubated with IL-2, the cell population was expanded in vitro and demonstrated cytotoxic activity. After administration of this immunotherapy extensive lymphoid infiltrates can be seen in tumors in conjunction with tumor destruction. Lymphokine activated killer cells (LAK) incubation of lymphocytes in IL-2 results in the generation of cells capable of lysing fresh tumor cells in short term 4 hour cytotoxicity arrays. When these cells were re-injected in patients with additional IL-2, tumor regression was observed. Subsequent studies demonstrated that the response rate and survival in response to high dose IL-2 alone was no different from that of IL-2 with LAK cells.[29, 30]

In a follow-up report of 283 patients who had received high dose IL-2, the 134 melanoma patients had 9 complete remissions (7%), 14 partial remissions (10%), for an overall response rate of 17%.[31]

Immunotherapy differs in its mode of action from conventional chemotherapy.

1. A response may take a long time to develop - particularly with interferon in keeping with the time course of cellular immunity.
2. Stable disease may exist for a long period as modulated host immunity holds tumor in immunological check.

Table 5. Strategy for the Identification of Human Tumor-Regression Antigens

1. Grow tumor-infiltrating lymphocytes (TIL) from patients with metastatic cancer and identify TIL that selectively recognize the autologous cancer in vitro, on the basis of assays of tumor cell lysis or cytokine secretion.

2. Administer these TIL to the autologous cancer patient and identify TIL that can mediate the regression of cancer in that patient.

3. Use these TIL to clone the gene(s) that encode the antigen(s) recognized by the TIL.

4. Determine whether the genes encode cancer regression antigens by:

   (a) Immunizing patients using these genes or gene products and determining whether the regression of growing cancers can be induced.
   (b) Sensitizing lymphocytes in vitro against the antigens encoded by these genes and determining whether adoptive transfer of these sensitized lymphocytes to patients can mediate the regression of growing cancers.

Reference 28
THE CANCER JOURNAL from Scientific American

Tumor Infiltrating Lymphocytes

The search for immune cells with improved therapeutic efficacy led to the discovery of a class of T lymphocytes with unique antitumor activity called tumor infiltrating lymphocytes (TIL).[28]

TIL cells circulate and specifically infiltrate into growing tumors. TIL can be grown in IL-2 by cultured single-cell suspensions obtained from tumors.
In vitro TIL cells specifically lyse the tumor from which they are derived and not other tumors. They are unique in the ability to identify cancer associated antigens in vitro. Human TIL can be both CD4+ and CD8+. Specific secretion of interferon gamma by TIL appears to be the best in vitro correlate of the in vivo antitumor effectiveness of TIL. A study of the lytic recognition of a variety of HLA-type melanomas by TIL demonstrated that melanomas from patients that share MHC antigens are often cross recognized by TIL as would be expected for an MHC restricted T cell reaction. In these studies it was found that the HLA-A2 class I determinant was frequently used as an MHC restriction element in the recognition of melanoma antigen. Presence of shared tumor antigen provided the possibility that these antigens may be used for active immunization against their own tumors. Libraries of cDNA have been prepared from human melanomas and are being transfected into non-antigen expressing autologous lines or tumor formed fibroblast lines in an attempt to identify the antigen recognized by TIL.

IL-2 Toxicity

Large doses of IL-2 can be associated with substantial side effects - The major toxicity appears to result from increased membrane permeability that leads to fluid and colloid loss into visceral organs and soft tissues.

TABLE 18-21. Toxicity Associated with the Administration of High-Dose Interleukin-2 Alone in 447 Consecutive Treatment Courses*

Category	*Toxicity (% of Courses)*
Systemic	Malaise† (14.5), chills† (18.3), pruritus† (8.9), weight gain >5% body weight (70.9), edema causing neurovascular compression (0.9)
Hemodynamic	Hypotension requiring pressors (52.1)
Cardiac	Arrhythmia (6.5), angina (1.1), myocardial infarction (0.4)
Pulmonary	Respiratory distress (4.0), intubation (2.9), pleural effusion requiring thoracentesis (1.1)
Renal	Oliguria (26.1), elevated creatinine (2.1–6 mg/dL = 62; >6 mg/dL = 6.3)
Gastrointestinal	Mucositis† (0.4), nausea/vomiting† (38.0), diarrhea† (30.2)
Hepatic	Hyperbilirubinemia (2.1–6 mg/dL = 62.4; >6 mg/dL = 21.9)
Neurologic	Disorientation (13.9), somnolence (4.9), coma (2.2)
Hematologic	Anemia requiring transfusion (21.0), Thrombocytopenia (20,000–60,000/μL = 29.3, ≤20,000/μL = 3.6)
Infectious	Infection (4.0), line sepsis (2.0)
Death	Treatment related (0.7)

* 283 patients with metastatic melanoma or renal carcinoma
† Grade III and IV toxicity only
(Schwartzentruber DJ. Biologic therapy with interleukin-2: clinical applications. In: DeVita VT, Hellman SH, Rosenberg SA, eds. Biologic therapy of cancer, ed 2. Philadelphia: Lippincott-Raven, 1995:235)

In an effort to decrease the potentially severe toxicity of bolus intravenous therapy with IL-2, alternate dosing schedules have been evaluated. Continuous infusion IL-2 has been used with only modest reduction in toxicity.[32] Subcutaneous administration of low-dose IL-2 is clearly less toxic but overall response rates vary and no consensus has been reached regarding its use. One rationale for the use of low-dose IL-2 is that the profound toxicity from high-dose IL-2 is from the release of secondary, pro-inflammatory cytokines. NK cells account for much of the secondary cytokine release. NK cells consistently express intermediate affinity IL-2 receptors whereas T-cells express high affinity receptors after antigen stimulation -- thus the T-cell IL-2 receptors are occupied and saturated prior to the NK cells -- which cause toxicity without therapeutic effect. [33, 34]

IL-2 and Interferon

The combination of the cytokines interferon and interleukin-2 is theoretically appealing because interferon alpha upregulates MHC class I molecules on tumor cells and can synergistically activate immunologic effector cells with IL-2.

In phase II trials the combination of cytokines yielded an average response rate of 22% (10-41% range) with duration of response ranging from three months to greater than 36 months.[35, 36]

In the only published randomized phase III trial comparing high-dose IL-2 (6 MU/m^2 IV bolus Q8 hr x 5 days) to IL-2 (4.5 MU/m^2 IV Bolus Q8 hr. x 5 days) plus interferon α IV (3 x MU/m^2) there was no difference in median survival between the two groups (10.2 mos. vs. 9.7 mos.)[37]

	N	PR	CR	Median Survival
IL-2	44	2	0	10.2 months
IL-2 + IFN	41	4	0	9.7 months

The trial was terminated early due to poor response. Of note, toxicity was the same in both arms.

Cytokines and Cytotoxic Agents: Chemoimmunotherapy

Patient responses to chemotherapy in melanoma do not predict for response to biologic response modifiers. There appears to be no clinical cross-resistance. This is likely due to the different mechanisms of cytotoxicity.[38] Theoretical concerns that chemotherapy might impair the immune mediated response in vivo appeared unwarranted as DTIC and cisplatin do not reduce IL-2 mediated induction of cytotoxic effector cells or the induction of secondary cytokines. Results of two recent reports of chemoimmunotherapy are summarized below.

The MD Anderson group employed two regimens.[39] In the first regimen the chemotherapy (cisplatin/vinblastine/DTIC) and biologic therapy (IL-2 9MU/m^2/day x 4 day CIV; IFNα 5 MU/m^2 day x 5 day SC) were given sequentially. In the second regimen they were given concurrently. (Table III)

In the Salpêtriere Hospital the patients were given cisplatin followed by IL-2 18 MIU/M2/day CIV and IFNα 9 MU/SC TIW with or without tamoxifen on three different schedules. the results are indicated in the table below.[40]

TABLE III
Number of Patients (%)

		N	CR	PR	Overall	Durable CR
M.D. Anderson	Sequential	62	13 (21)	23 (38)	36 (59)	7 (11)
	Concurrent	53	11 (20)	22 (42)	33 (62)	5 (9)
Salpêtriere		127	13 (10)	49 (39)	62 (49)	9 (7)

A phase III EORTC trial in which 138 patients with advanced melanoma (86% with visceral metastases) were randomized to IFNα (10MU/m^2 day 1-5) and IL-2 (decrescendo IV day 4-9) with or without a single dose of cisplatin on day 1. The response rate in the IFN/IL-2 group was 18% and in the IFN/IL-2/cisplatin group was 35%. The overall survival was nine months, identical in each group.[41]

Other phase III studies examining the role of biologic therapy plus chemotherapy are currently being conducted.

Active Specific Immunotherapy

Active specific immuntherapy refers to the use of tumor associated antigens to immunize a tumor bearing host in order to effect the rejection of that tumor.[42] The mere recognition of tumor antigens is not sufficient to eliminate tumor cells because antigenicity does not confer immunogenicity. Most tumor antigens are weakly immunogenic. It is important to note that patients with extensive disease have already been exposed to an abundance of antigens. Multiple methods have been used in a myriad of studies to augment the immunogenicity of melanoma vaccines. Following is an outline of some of the varied approaches which have been undertaken to develop vaccines directed agianst melanoma cells and the antigens which they express. Vaccines have been developed from autologous tumor cells, allogenic tumor cells, tumor cell lysates prepared in different ways including viral oncolysates, ganagliosides and modified tumor antigens.

As indicated in the studies cited below the focus has predominantly been on stimulation of cellular immunity, however antibody responses have also been evaluated. Regarding ganglioside vaccines, stimulation of humoral immunity is the major focus.

Autologous Melanoma Vaccine

Before the discovery of tumor antigens common to many different melanoma cells, it was thought that melanomas had specific antigens unique only to the individual and not shared by other tumors of the same melanoma. Therefore, it seemed necessary to vaccinate patients with their own tumor cells (autologous vaccine). Through this method the best match between HLA phenotypes could also be obtained.

David Berd, in conjunction with colleagues in Italy, has studied an autologous tumor vaccine linked to the hapten dinitrophenyl (DNP) based on previous work with TNP-Hapten associated vaccine in mice.[43, 44] The addition of the hapten DNP is an attempt to make the autologous cell more "foreign" and thereby increase immunogenicity. Responses were seen in 5/40 patients with metastatic disease, mainly in soft tissue, lymph nodes and skin. In a single arm trial examining 62 patients with surgically resected lymph node disease, an improvement

in disease free (45%) and overall (58%) 5 year survival was seen in patients treated with the hapten enhanced autologous vaccine.[45]

Eliciting a DTH response to autologous tumor cells was a necessary but not sufficient factor in responding to the vaccine. In analyzing the data, the authors observed that patients with haplotpye $A3^{+}A2^{-}$ had an overall worse response, while elderly patients had a better response. The authors postulated it may have been related to autoimmunity. They also noted inflammation in recurrent tumor sites in patients previously treated with tumor vaccine. Inflammation of recurrent tumor was associated with a prolonged survival. However, the magnitude of DTH response to DNP modified melanoma cells was not predictive of clinical outcome.

Others have demonstrated cloned expansion of T cells expressing peculiar TCR B-chain variables (TCRBV) gene families at the tumor site of some patients treated with DNP haptenized vaccine suggesting that the vaccine does in fact elicit a specific immunologic response.[46]

The need for accessible tumor nodules of sufficient size (>3 cm in diameter) to provide tumor cells in adequate numbers to serve as an autologous immunogen make it impractical to use this method on a large scale.[47] Another limitation to the broad use of this approach, the inherent weak immunogenicity, of autologous tumors, may be overcome by haptenization.

Ganglioside Vaccines

Gangliosides are membrane bound glycosphingolipids containing sialic-acids which are found in high concentration in malignancies of neutral crest origin such as melanoma. These molecules play an important role in cell-cell interactions. Most are found in the plasma membrane, with their carbohydrate moieties pointing outward, where they are ideally situated to act as cell surface antigens.[48] Three of these gangliosides, GM2, GD2 and GD3 were identified as the most immunogenic of melanoma antigens[49] when injected into mice, or in patient studies, where antibodies to GM2, GD2 and DG3 resulted in some clinical responses. Overall the gangliosides are poor immunogens and the induction of antiganglioside antibodies is difficult.

Gangliosides have several characteristics for cell surface antigens to be targeted by antibodies: high and homogenous expression in tumor, minimal expression in normal tissues, little or no soluble form or accessibility to the circulation. Since the antigens are not sufficiently immunogenic or because antigen negative cells emerge, adjuvants are required to make immunization against the antigen more effective.

Ganglioside vaccines have demonstrated an IgM response in most patients but this is of short duration with a general lack of IgG response. There is a lack of stimulation of T cell mediated immune response in such trials.

Livingston, et. al.,[50] reported on 122 patients. Stage III patients were free of disease after resection. They were randomized to either GM2 vaccine plus BCG vs. BCG alone. All patients received a pretreatment low dose of cyclophosphamide, in an effort to reduce suppresser activity: IgM antibodies to GM2 were detected in 50/58 patients treated with GMs/BCG vs. 7/64 treated with BCG alone. There was an improvement in survival in patients who were antibody positive. When patients with pre-existing GM2 antibodies were excluded from analysis then the GM2/BCG group had an improved disease free survival of 23% and overall survival of 14%, however, when all patients were included in this analysis, the difference in survival were no longer statistically significant.

Efforts to improve the immunogenicity of the GM2 vaccine include conjugating the GM2 to the carrier protein keyhole limpet hemocyanin (KLH) and adjuvanted with QS-21. A bivalent vaccine with GM2/GD2 with KLH and QS-21 is also being tested. Similarly, GD3 has been conjugated to KLH and adjuvanted with QS-21 eliciting a strong IgG response in immune models[51]. GD3 has also been bound to liposomes in murine models to improve immunogenicity.[52]

Allogeneic Tumor Cell Vaccine

Allogenic tumor cell vaccines have several theoretical advantages. They may present more than one antigen for potential immunogenity; patients are not required to have a certain amount of tumor in place; uniformity of the vaccine can be carefully controlled and vaccine can be developed in large quantity.

The polyvalent melanoma cell vaccine (PMCV) developed and extensively studied by Morton et. al., is derived from three melanoma cell lines.[53] This is a living irradiated vaccine which induced a specific antimelanoma immune response with increased humoral antibodies to cell surface antigens on allogeneic and autologous tumor cells and to well defined melanoma associated tumor antigen such as GM2, CD2, UTAA and MAGE-1. Cellular immunity was activated as judged by a concomitant increase in cytotoxic T lymphocyte activity and mixed tumor lymphocyte reaction to allogeneic and autologous melanoma cells as well as induction of delayed type hypersensitivity to the melanoma vaccine.

In patients with metastatic melanoma who underwent cytoreductive surgery for visceral metastases, those who had follow-up adjuvant therapy with PMCV had an improved survival compared to 76 patients who received other post-operative adjuvant therapy.[54]

For patients with completely resected skin, subcutaneous or lymph node metastases those who received PMCV as an adjuvant also demonstrated an improved survival as compared to patients who received other post-operative adjuvant therapy.

TABLE I

Stage IV Patients With Visceral Metastases - Survival After Surgery

	N	Median	5-year
Surgery & PMCV	46	32.3 months	33%
Surgery & Other Adjuvant	76	12.6 months	10%

Stage IV Patients With Skin Subcutaneous or Lymph Node Metastases - Survival After Surgery

	N	Median	5-year
Surgery & PMCV	44	37.5 months	39%
Surgery & Other Adjuvant	54	20.5 months	19%

TABLE II

Stage III Patients Post Operative - Disease Free Survival

	N	Median	5-year	10-year
Surgery & PMCV	283	>80 months	52%	47%
Surgery & Other Adjuvant	1474	24.3 months	36%	31%

Stage III Patients Post Operative - Overall Survival

	N	Median	5-year	10-year
Surgery & PMCV	283	>90 months	53%	49%
Surgery & Other Adjuvant	1474	35.1 months	39%	33%

The strength of DTH skin test response to PMCV antigen as well as generation of IgM antibodies was correlated with improved five year survival rates.

In stage III (lymph node involved) patients who had complete resection, the use of PMCV as adjuvant was correlated with improved disease free and overall survival in comparison to historical controls who received other post-operative adjuvant therapy. (Table II)

This year a new international, multi-institutional, randomized control trial will be opening to accrual to determine if stage IV patients who underwent complete resection will have an improved survival when given PMCV plus BCG vs. BCG alone. Stage III patients will be randomized to PMCV vs. high dose interferon as a post-operative adjuvant therapy. The University of Texas Southwestern Medical Center will be one of the study sites.

Allogeneic Tumor Cell Lysate Vaccine

Using a vaccine composed of mechanically disrupted melanoma cells from two cell lines, together with the adjuvant DETOX (mixture of M. phlei cell wall skeletons, monophosphoryl lipid A, squalene), Mitchell et. al., treated patients with disseminated melanoma.[55]

One half of the patients also received low-dose cyclophosphamide prior to vaccination. Increases in precursors of CTL against one of the two melanoma cell lives in the lysates was noted in 50% of patients two to six weeks after immunization. CTL clones were derived from immunized patients with strong reaction against autologous and allogeneic melanoma cells.

Complete responses and partial responses were noted in some patients, all of whom had an increase in CTL in the blood. Serum antibodies developed in nearly 50% of patients but did not correlate with clinical response. Five complete responses (CR) and 15 partial responses (PR) (19%) were seen in 106 patients with regression in several metastatic sites. The median duration of response on therapeutic vaccine treatment was 21 months.[42]

A multicenter trial using the mechanical lysate vaccine (Melacine) in 139 patients showed lower objective response rates (3% CR; 5% PR) but 23% of patients has stable disease for more than six months. Median survival for the 120 patients actually treated was 23 months.

A phase III trial comparing Melacine to four drug chemotherapy in two groups of 70 patients found survival to be equivalent in both groups with far fewer reportable adverse events attributed to the vaccine.

Bystryn et. al., has treated over 400 patients with a partially purified, polyvalent melanoma antigen vaccine which is prepared from material shed into culture from four separate lines of melanoma cells.[56] In a report of 82 such patients, a relationship was noted between the stimulation of an antibody response to melanoma, the strength of that response, and improved prognosis. Five year survival was 71% for the 32 patients with vaccine induced antibody (to at least one antigen) and 44% for the 50 non-responders. A statistically significant and clinically meaningful correlation between strong vaccine-induced delayed-type hypersensitivity response and improved median disease free survival was noted. [57]

Viral Oncolysates

Virus induced oncolysis of tumor cells can enhance the immunogenicity of those cells. This observation led to the development of therapeutic vaccines composed of virally transformed tumor cells. Viral lysates have been induced by Newcastle disease virus, vaccinia virus and influenza virus.[58] Evidence of tumor shrinkage in skin nodules or lymph nodes was seen in seven out of 13 patients with Newcastle disease virus induced melanoma cell lysate.[59]

At the Sydney Melanoma Unit, 96 patients received vaccinia melanoma cell lysate (VMCL). The VMCL was prepared from a single allogeneic melanoma cell line and was given in a post-operative adjuvant setting. The median survival was five years as compared to two years in historical controls.

Subsequently 102 patients received VMCL plus cyclophosphamide, which served to diminish the improvement in survival.[60]

VMO

Vaccinia Melanoma Oncolysate (VMO) was evaluated in a phase III trial which involved 250 patients.[61] These patients were post surgical and had one to five lymph nodes positive for melanoma. Patients were randomized to receive either VMO vs. placebo consisting of live vaccinia virus. The VMO was prepared from four melanoma cell lines which expressed melanoma-associated antigen, such as MAGE-1, fetal associated antigen, urinary tumor - associated antigen, GD3, GD2 and GM2. There was no statistically significant difference in the disease free interval or overall survival, although there was a 10% difference in overall survival at four years. It was also noted that a subset of male patients aged less than 57 with one to five lymph nodes had a 25% improved survival with the VMO (not statistically significant).[62]

Antiidiotype Vaccine

Antiidiotype vaccines rely on a property of a small subset of antibodies to mimic the melanoma antigen, either by their structure or effect on the immune system. Antibody 1 is generated against the antigen. Antibody 2 (antiidiotype) is generated by immunizing an animal with antibody 1. A subset of antibody 2 reacts with the hypervariable region of antibody 1, and a subset of that group mimics the antigen. Antiidiotypes must be chosen carefully or risk having no effect, or suppressing immunity.[63]

Twenty five patients with advanced melanoma were treated with antiidiotype vaccine I-Mel-2 together with the potent adjuvant SAF-m. I-Mel-2 is a murine monoclonal antiidiotype antibody that mimics an epitope of MPG (high molecular weight melanoma associated antigen) in its activity. Elevated titers of human antimurine antibodies and anti-I-mel-2 antibodies were associated with clinical antitumor response. Objective regression was seen in only one patient.[64]

Recent Reports

Two articles, published in the March 1998 issue of the journal, Nature Medicine, illustrate different approaches to vaccination in melanoma. Each demonstrates an attempt to enhance the CTL immune response based on manipulation of antigen recognition.

A European group, led by Nestle et al.[65] processed dendritic cells (DC), known to be the most potent of the APC (antigen-processing cells), in vitro with cytokines such as IL-4 and GM-CSF to promote their growth. These dendritic cells were pulsed with autologous tumor lysate or a cocktail of tumor antigens (based on the patient's HLA haplotype) which bind to the HLA-A1 or HLA-A2 molecule. The cells were also pulsed with a globular protein antigen, keyhole limpet hemocyanin (KLH) which has to be taken up, processed and loaded on MHC class II molecules by dendritic cells for effective presentation to helper T-cells. Repeated vaccination led to a sustained delayed type hypersensitivity (DTH) to the neo and helper-antigen KLH indicating specific memory T cell production. Ex Vivo peptide pulsed DC's were injected intradermally and clearly enhanced the DTH reaction in 15 out of 16 patients. There was a correlation between DTH reactivity and clinical activity, as well as detection of peptide specific CTL's. Large numbers of $CD8^+$ T cells at the DTH challenge sites were expanded in vitro and tested for peptide specific cytotoxicity. This demonstrated cytotoxicity to Melan-A and GP-100 but not tyrosinase. In evaluating the tumor response in these 16 patients, in whom tumor burden was low, two complete responses were seen in patients with cutaneous lesions alone. There was significant partial response in metastatic sites in four other patients.

One of 6 patients expressing HLA-A1 haplotype and immunized with MAGE -1 and MAGE-3 responded to DC vaccination. Two of 6 patients immunized with HLA-A2 peptide exhibited a complete response and partial response. Four patients were immunized with DC pulsed with autologous tumor lysatse. Two of whom had a response (1cr;1pr), these results are encouraging and will require further investigation.

The study from Rosenberg et al[66] used tumor infiltrating lymphocytes (TIL) grown from metastatic melanoma nodules to identify melanoma antigens associated with tumor rejection. Two differentiation antigens, MART-1 and GP 100 (whose recognition was HLA-A2 restricted) were identified. Epitopes of these antigens were evaluated for their binding affinity to the HLA-A2 molecule with particular attention to MHC binding anchor residues. Synthetic peptides with 1 or 2 amino acid substitutions were evaluated for their binding capacity.

A modified g209-217 peptide (a gp100 epitope) referred to as g209-2M, in which a threonine in the native peptide was replaced by a methionine had a greater binding affinity to the HLA-A2 molecule and was found to generate a greater melanoma cytotoxic T-lymphocyte response in vitro.

In this study, patients with HLA-A2 haplotype were studied. Two out of 8 patients who received the native g209-217 peptide developed reproducible response to the peptide as compared to 10 out of 11 patients who received the modified g209-2M peptide. Nine patients received the g209-217 peptide in Incomplete Freund's-adjuvant (IFA). The emulsification of peptide in adjuvant may provide prolonged exposure to antigen and may activate non-specific inflammatory cells and bring APC's to the site of immunization.
Eleven patients received the g209-2M peptide in IFA, 31 received g209-2M peptide in IFA plus systemic IL-2. (Nineteen patients with metastatic melanoma received the g 209-2M peptide in IFA followed by IL-2. Only 3/19 developed immune reactivity in peripheral blood mononuclear cells (PBMC) as compared with 10/11 patients who received 209-2M peptide in IFA without IL-2. Twelve additional patients received g209-2M peptide for 2 cycles before receiving g209-2M with IL-2. One out of 9 patients receiving the g209-217 peptide experienced a tumor regression. None of the 11 patients receiving g209-2M in IFA responded. Of 19 patients who received g 209-2M concomittant with IL-2, 8 patients exhibited an objective tumor regression(42%). Of the 12 patients who received g 209-2M in IFA followed by g 209-2M in IFA plus IL-2, 5 responses (42%) were seen. This is substantially higher than the response seen with IL-2 alone (15-17 %). Of note, some of the regressions noted were in patients with bulky metastatic disease. Most lesions were cutaneous, subcutaneous, lymph node or lung; there was one reported regression of a brain lesion.

Exposure to g209-2M peptide in IFA induced high levels of tumor reactive cells but this did not correlate with clinical response. Thus the addition of IL-2 appears to have increased the immunizing capacity of MHC class I reactive antigen.

Further studies are being undertaken and utilizing several peptide injections at once. Another study is evaluating in vitro processing of PBMC in the presence of g209-2M and IFA at IL-2 with adoptive transfer to autologous tumor bearing patients.

In Conclusion

The efforts to better understand the antigenic and immunogenic potential of melanoma cells have yielded greater insights into the mechanism of cellular immunology and tumor immunology overall. This in turn has allowed investigators to develop more specifically targeted immunologic therapies. The clinical relevance and overall efficacy of these varying approaches still requires extensive evaluation. Nonetheless it is clear that some patients have already benefitted from the biologic therapy of melanoma.

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