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STATE-OF-THE-ART IN THE MANAGEMENT OF CANCER

MALIGNANT MELANOMA, PART II

MEETING COVERAGE

OPPORTUNISTIC INFECTIONS IN CANCER PATIENTS

A REPORT FROM THE 35TH INTERSCIENCE CONFERENCE ON ANTIMICROBIAL AGENTS AND CHEMOTHERAPY (ICAAC), SAN FRANCISCO, CALIFORNIA; SEPTEMBER 17-20, 1995

TECHNOLOGY UPDATE

NUCLEAR MEDICINE

STATE-OF-THE-ART IN THE MANAGEMENT OF CANCER

MALIGNANT MELANOMA, PART II

NEW DRUGS AND MODALITIES IN DEVELOPMENT FOR THE TREATMENT OF MALIGNANT MELANOMA

- Although early stage melanoma is curable by surgical excision, metastatic melanoma is a refractory malignancy.
- Human melanoma tumor cells exhibit high resistance independently of the chemotherapeutic chosen for treatment; only 5%-10% of patients treated with any regimen experience durable responses.
- Potential worldwide markets for preventative and/or therapeutic vaccines could reach $150 million annually, as forecast in Exhibit 1.
- Although conventional chemotherapeutics are in development to treat malignant melanoma, most of the current emphasis is on various immunotherapies (see Exhibit 3).
- New findings regarding the function of the immune system will have important ramifications on the design and clinical use of cancer vaccines against melanoma and other cancers.
- Abstracts referenced in this article are from the 86th Annual Meeting of the American Association for Cancer Research (abbreviated as AACR95), the 31st Meeting of the American Society of Clinical Oncology (abbreviated as ASCO95) and the 38th Annual Clinical Conference on Advances in the Biology and Clinical Management of Melanoma, February 21-24, 1995, Houston, TX (abbreviated as ACC95).

IMMUNOTHERAPY APPROACHES FOR THE TREATMENT OF MALIGNANT MELANOMA

Human primary malignant melanoma is highly immunogenic, often eliciting a host response of infiltrating T lymphocytes suggestive of tumor antigen-induced immunity. The significance of this infiltrate is not fully understood but it was shown to correlate with a more favorable prognosis. In contrast, metastatic melanoma typically is not accompanied by inflammation and, consequently, it is highly lethal.

When appropriately stimulated melanocytes and malignant melanoma cells synthesize and release a variety of cytokines that upregulate immune and inflammatory responses, including interleukin-1α (IL-1α), IL-1β, IL-6, tumor necrosis factor-α (TNF-α) and chemokine IL-8. Among these IL-6 appears to be particularly relevant in enhancing a host immune response. In addition, melanoma cells release inhibitory cytokines such as the cytokine synthesis inhibitor IL-10, transforming growth factor-β (TGF-β) and IL-1 receptor antagonist. Actually IL-10 can be used as a measure of tumor load because tumor progression tracks serum levels of IL-10.

Melanoma cells also produce pro-opiomelanocortin peptide hormones such as α-melanocyte stimulating hormone (α-MSH) that was found to down-regulate IL-1 and up-regulate IL-10 (Luger, Thomas A, etal, ACC95, p 4). It also appears that early stage primary melanomas are sensitive to growth inhibitory cytokines whereas late stage primary and metastatic tumors become resistant to these factors. Multi-cytokine resistance in advanced disease may be also accompanied by cytokine "switching", i.e. conversion of an inhibitory factor into a stimulator of cell growth (Mintz B, ACC95, pp 28-29).

Various immunotherapy regimens are being attempted to treat/prevent metastatic melanoma including active (specific and non-specific), adoptive and passive immunotherapy. Generally, vaccines have not been effective in the treatment of melanoma, in particular, and cancer, in general. One of the reasons is that vaccine approaches rely more on empirical observations rather than a clear understanding of the workings of the immune system. New findings constantly challenge accepted dogma as researchers delve deeper into the inner workings of this incredibly intricate machine.

Despite the emergence of novel antigens associated with tumor formation, the host's immune system fails to recognize these tumor cells as non-self, allowing them to grow. Recent data suggests that dendritic cells, cells that display foreign proteins on their surface to activate killer T cells, must also provide costimulatory or other signals for full T cell activation. This initiates the cascade of

Exhibit 1
Estimated Potential Worldwide Markets of Therapeutic and Prophylactic Melanoma Vaccines

Vaccine Type	Recipients* at 5th Year (#)	Manufacturer's Revenue per Regimen ($)	5th Year Potential Worldwide Markets ($ 000)
Therapeutic vaccine, administered once	20,000	2,500	50,000
Therapeutic vaccine with annual booster	100,000	2,500 plus 1,000 booster	130,000
Prophylactic vaccine administered once	80,000	500	40,000
Prophylactic vaccine with annual booster	400,000	500 plus 350 booster	152,000

** Based on worldwide melanoma populations estimated in FO, V1 #5, p 3.*

events resulting in an immune response. When dendritic cells display only small amounts of foreign or tumor antigen to the T cells, however, they may not provide a sufficient activation signal. In the case of early oncogenesis, a small tumor is not "seen" by the immune system, and its specific antigens are tolerated until it becomes too large to destroy. This theory may explain why vaccines using tumor antigens have not proven very effective. Administered to patients with widespread, resistant disease, they do not elicit a sufficiently strong immune response because the system has long stopped perceiving the tumor as foreign. Such findings may prompt the use of vaccines early in the course of the disease to increase the immune system's sensitivity to small amounts of tumor antigens, so it mounts an aggressive response. Also, this may support a different approach to vaccine delivery, favoring repeated booster doses at regular intervals. Some evidence suggests that dendritic cells are induced to initiate a productive immune response in part by heat shock proteins, already being developed as immunotherapeutic agents.

Systemically-delivered Cytokines as Immunomodulators

Cytokines can be potent mediators or modulators of immune response. When evaluated *in vitro* cytokines exhibit varying degrees of effectiveness as monotherapies and as adjuvants to traditional chemotherapy. This observation has led to clinical trials of various cytokines, alone or in combination, in the treatment of malignant melanoma.

Systemically-delivered Interferons

In studies with interferons (IFN), IFN-β and IFN-γ proved to be more antiproliferative than IFN-α in a dose-dependent fashion in all melanoma cell lines. However, the ability of IFNs to improve cytotoxicity of chemotherapeutic agents has been limited. Pre-incubation of melanoma cells with IFN as well as exposure to IFN after-incubation with chemotherapeutics showed mainly additive effects. Therefore, combinations of chemotherapeutic agents with IFN may provide additional therapeutic benefit, but is unlikely to change the overall high chemoresistance of human melanoma cells (Schadendorf D, etal, Melanoma Research, 1994 Aug, 4(4):243-9).

Interferon-α (IFN-α) 2b (Intron-A; Schering-Plough) has been approved for the treatment of resected malignant melanoma (see FO, V1 #5, p 126). Response rates to IFN-α monotherapy are about 15%, with only about 1%-5% lasting over 5 years. Combination therapies using IFN-α that may produce responses in up to 25%-40% of those treated, are currently under investigation.

IFN-α 2b combined with Melacine, a vaccine under development by Ribi ImmunoChem Research (Hamilton, MT), has shown activity as an adjuvant in patients with disseminated melanoma who did not respond to previous treatment with this active specific immunotherapy modality. While the specific mechanism of activity of the combination therapy is undetermined, it is theorized that therapy with IFN may potentiate the activity of anti-melanoma cytolytic (or helper) T lymphocytes produced in response to Melacine therapy or it may directly affect a patient's melanoma tumor cells to make them more immunologically visible to the immune system. Using IFN to increase antigenic visibility of tumor cells may have enabled successful "salvage therapy" in patients who had responded to Melacine therapy but whose tumors were not sufficiently visible for the Melacine response alone to be effective. In May 1995 Ribi received FDA approval to initiate a phase III 30-center clinical trial of 300 patients with stage IV (disseminated) melanoma to compare the effects of Melacine (with Detox adjuvant) in combination with IFN-α 2b. The study will compare Melacine plus IFN against a control arm of IFN monotherapy; endpoints are overall survival as well as disease-free survival, quality of life, clinical response rates (tumor regression) and safety and toxicity.

IFN-α 2a/retinoid combinations demonstrated synergistic antiproliferative and differentiating effects in preclinical tests in some hematologic and solid tumor models. The observation that combination of 13-cis retinoic acid (13cRA) and IFN-α can induce regression in advanced tumors is very important because both bind to specific receptors and change gene expression. Clinical trials of this combination, however, did not demonstrate effectiveness against melanoma (Eisenhauer EA, etal, Leukemia, 1994, 8 Suppl 3:S38-41).

IFN-γ. A randomized trial of IFN-γ, undertaken by the Southwest Oncology Group (SWOG; San Antonio, TX), was discontinued after interim analysis indicated an adverse effect.

Systemically-delivered Interleukins

Although large scale clinicals are ongoing to assess the immunomodulatory activities of interleukins in the treatment of malignancies, the process has been hampered by the fact that key mechanisms of interleukin-induced immunomodulation are not clearly defined and the methodology for assessing immunostimulatory effects of interleukins is not well established. However, two malignancies, renal cell carcinoma and malignant melanoma, have been successfully treated with IL-2 in selected cases. Also, use of interleukins as therapeutic agents is marred by their serious side effects. Several approaches, including novel delivery systems, are currently under investigation that may diminish some of the side effects of IL-2.

Interleukin-2 (IL-2) is a potent immune-cell stimulant that is toxic in high doses. It induces the secretion of pro-inflammatory cytokines such as IL-6, IFN-γ and tumor necrosis factor-α (TNF-α). The effects of IL-2 include:

- activation of the body's immune system against certain cancers
- systemic inflammation resulting from oxidative injury to endothelial cells
- toxic side-effects, including low blood pressure (hypotension), organ damage (heart, kidneys, lungs, CNS), anemia and a clinical syndrome similar to SIRS (systemic inflammatory response syndrome)

In May 1992, interleukin-2 (IL-2) was formally approved by the FDA for use in the treatment of cancer, based on its activity in metastatic renal cell carcinoma. IL-2 alone or in combination with activated lymphocytes or other cytokines has significant anti-tumor activity against renal cell carcinoma and melanoma with response rates of 15-35%, some of which are quite durable. However, IL-2 therapy is a complex procedure and, despite its clinical efficacy, it is shunned by many clinicians because of its perceived toxicity. Also, randomized trials have failed to establish treatment guidelines regarding optimal administration methods (high-dose bolus or continuous infusion) or combination regimens (IL-2 plus IFN or IL-2 plus lymphokine-activated killer (LAK) cells versus IL-2 alone (Dillman RO, Cancer Biotherapy, 1994 Fall, 9(3):183-209). Among 134 patients with metastatic melanoma participating in a trial to determine the efficacy of high-dose (720,000 IU/kg every 8 hours for a maximum of 15 doses per cycle) bolus IL-2, only 9 (7%) achieved complete regression and another 14 (10%) experienced partial regression. Complete regressions lasted for up to 91 months (Rosenberg SA, etal, JAMA, March 23/30, 1994-Vol 271, No. 12, pp 907-913).

IL-2 analogs are under development to mitigate IL-2 toxicity which is believed to be mediated by such secondary cytokines as IL-1, TNF, and IFN-γ, which are produced by the patient's IL-2-stimulated peripheral blood mononuclear cells (PBMCs). IL-2 analogs that preferentially bind the intermediate-affinity IL-2 receptor (IL-2R) induce substantial LAK cell activity despite significant reduction of secondary cytokine production (Heaton KM, etal, Annals of Surgical Oncology, 1994 May, 1(3):198-203).

Cell Therapeutics (Seattle, WA), a privately held company, has been clinically evaluating Lisofylline, its lead adjunctive oncology product, as a means of curtailing the systemic inflammatory side-effects of IL-2 therapy, including multi-organ dysfunction. Animal studies of Lisofylline have shown it to be a potent blocker of specific molecular species of phosphatidic acid (PA), an intracellular lipid second-messenger that controls the production of several hematopoietic inhibitory cytokines such as TGF-β, TNF-α and IFN-γ. Lisofylline was clinically evaluated in three phase I trials of more than 90 cancer patients who received over 8,000 doses and was deemed safe, well tolerated, and capable of preventing toxicity from multiple forms of cancer treatment, including promoting rapid recovery of white and red blood cells as well as platelets. Lisofylline may accelerate the recovery of all three types of blood cells following high-dose chemotherapy by preventing the production of these inhibitors of hematopoiesis. As of mid-1995, results of a multi-center phase II/III clinical trial of Lisofylline indicated a significant decrease in systemic inflammation and toxicity following concurrent intravenous administration of high-dose IL-2 in cancer patients. In this 53-patient, double-blinded, placebo-controlled trial, Lisofylline was administered intravenously to patients treated with IL-2 for renal cell cancer or malignant melanoma. IL-2 was delivered at a dose of 600,000 IU/kg/day in 3 divided daily doses for a projected total of 14 doses. After a 10-day rest period, the regimen was restarted for an additional 14 doses. Lisofylline or placebo was given at a dose of 1.5 mg/kg every 6 hours over the same treatment period as IL-2. The primary

efficacy endpoints were evaluated on the day of the scheduled eighth IL-2, the "observation period." A significant increase in the percentage of patients able to receive full doses of IL-2 (70% versus 38%), and a significant decrease in the severity of organ toxicity (renal, neurologic, hypotension, grade-2 cardiac, pulmonary) in patients receiving Lisofylline compared to controls, were observed at the end of the first week of IL-2 therapy.

IL-2/IFN-α combinations are being evaluated by several groups with some encouraging early-stage results. However, long-term results with such combinations were disappointing in patients with metastatic cancer based on follow-up evaluation of a phase I/II study of 189 patients (82 had melanoma) treated with 379 courses from November 1987 through October 1990, overall 5-year survival rate was only 11%. Based on these findings investigators concluded that further studies of this combination treatment were not warranted (Marincola FM, etal, Journal of Clinical Oncology, 1995 May, 13(5):1110-22). Currently, however, many studies are ongoing assessing this combination.

IL-2/IL-1 β combinations have synergistic antitumor and myelostimulatory activities. In a phase I dose ranging study tumor regressions were observed in patients with colorectal cancer, melanoma, and renal cell carcinoma. The addition of even low-dose IL-1 β to IL-2 may be associated with potentially beneficial biologic activity; higher doses of IL-1 β may add potentially beneficial hematologic activity (Triozzi PL, etal, Journal of Clinical Oncology, 1995 Feb, 13(2):482-9).

IL-2/loxoribine combinations resulted in significantly greater inhibition of melanoma metastasis. R. W. Johnson Pharmaceutical Research Institute (Don Mills, Ontario, Canada) is developing loxoribine (7-allyl-8-oxoguanosine; RWJ-21757), a substituted guanosine analog, which is a B-cell-selective activator of the humoral immune response (Reitz AB, etal, Journal of Medicinal Chemistry, 1994 Oct 14, 37(21):3561-78). Loxoribine exhibits adjuvant activity for B cells, activates natural killer (NK) cells, and enhances the activation of LAK cells by IL-2. Loxoribine, given in a single injection of 2 mg inhibited melanoma B16 metastasis in mice as late as day 3 of tumor growth. The greatest inhibition (96%) was seen in mice when four injections of loxoribine were administered on alternate days, starting the day before tumor injection. Loxoribine also exhibits adjuvant activity. Mice immunized with both irradiated tumor cells and loxoribine developed a significantly lower number of lung tumors when challenged by live B16 tumor cells, compared to mice injected with either vaccine or loxoribine. The degree of protection was dose-dependent. Loxoribine may be useful in tumor therapy as an immunomodulator or as an adjuvant for use with tumor vaccines (Pope BL, etal, Cancer Immunology, Immunotherapy, 1994 Feb, 38(2):83-91).

IL-4, a pleiotropic cytokine, exhibits various direct and indirect effects on B cells, T cells, monocytes/macrophages and eosonophils. It appears to act as a potent second signal in immune regulation, exhibits colony-stimulating activity, participates in immunoglobulin synthesis, increases certain cell surface proteins and is cytostatic against certain human tumors *in vitro*. Several phase I dose ranging clinical trials are currently under way at M. D. Anderson Cancer Center (Houston, TX) in patients with solid and hematologic malignancies (Markowitz AB, Cancer Inves 1994: 12 (Suppl 1: 4-5).

IL-12 (see FO, V1 #2/3, p 52)

Active Specific Immunotherapy-Tumor Cell Vaccines

Active specific immunotherapy (ASI) was attempted in melanoma as early as 1960. Originally, vaccines were based on autologous tumor cell preparations mixed with various adjuvants. Although initial results with these vaccines were dramatic with most patients experiencing early regressions, long-term response was disappointing. Since then considerable progress has been made in refining these vaccines and various ASI approaches, extensively tested in phase II clinical trials, were shown to alter the natural course of stage III and IV melanoma following surgical resection of nodal or distant metastases.

An autologous tumor cell vaccine modified by the hapten dinitrophenyl (DNP), under evaluation by researchers at Thomas Jefferson University (Philadelphia, PA), induced inflammatory responses in metastatic melanoma masses and sometimes resulted in tumor regression. Histologic examination have shown that these tumors were infiltrated with T lymphocytes (Sato T, etal, Clinical Immunology and Immunopathology, 1995 Jan, 74(1):35-43 and Berd D, etal, Cancer Immunology, Immunotherapy, 1994 Sep, 39(3):141-7).

Practical considerations, however, turned investigators' interest to allogeneic tumor cell vaccines that circumvent the need for autologous tumor cells. Allogeneic vaccines enable treatment/prevention of metastatic cancer long after removal of the primary melanomas. Various tumor antigens present on melanoma tumor cells have been identified (see Exhibit 2) and are currently being evaluated in various vaccine preparations.

A polyvalent allogeneic melanoma cell vaccine (MCV) containing high concentrations of six melanoma-associated antigens has been tested in phase I and II trials at the John Wayne Cancer Center (Santa Monica, CA) with promising results in stage IV metastatic melanoma. MCV induced cell-mediated and humoral immunity in approximately 80% of immunized patients and extended the 7.5-month median survival of stage IV melanoma patients to 23 months. A significant cell-mediated immune response to MCV is associated with a two fold to threefold increase in median and 5-year survival. Because the objective MCV response rate in patients with evaluable disease is only about 20%, it is theorized

Exhibit 2
Selected Antigens Associated with Melanoma

Markers/Antigens	Description
Antibody HMB-45	Used as an immunohistochemical reagent for melanoma; some benign lesions have been noted to show positive staining reactions with this reagent
MAGE family (MAGE-1 and MAGE-3) of class I MHC-restricted human melanoma associated tumor antigens	Neoantigens that are not expressed by normal melanocytes from which they are derived; also found exclusively in human adult testes tissue (van der Bruggen PC, et al, Science, [1991] 254:1643)
MART-1/Melan-A	May have application in novel immunotherapeutic strategies for treating patients with melanoma, including redirection of TIL specificity and bone marrow stem cell therapy (Cole DJ, etal, Cancer Research, 1995 Feb 15, 55(4):748-52)
Human endoglin (CD105), a member of the TGF-β receptor family	CD105 is an Arg-Gly-Asp (RGD)-containing molecule potentially involved in cell to cell interactions through binding to cellular integrins; immunohistochemical analyses demonstrate that CD105 is differentially expressed on normal and neoplastic cells of the melanocytic lineage; CD105 is the first RGD-containing protein identified on cells of the melanocytic lineage; it may play a relevant role in human melanoma by regulating the sensitivity of neoplastic cells to TGF-β and modulating cell-mediated host-tumor interactions (AACR95, Abs. 3814)
GM3, GD3, GM2 and GD2	Acidic glycolipids, gangliosides; may be tumor specific, in that they are not found in normal adult or fetal tissues; expressed on the cell surface of malignant melanoma; immunization with GM2 or melanoma cell vaccine induces IgM anti-GM2 and GD2 antibodies which have been shown to have anti-tumor effects (Takahashi, T, etal, AACR95, Abs. 2891)
Antigens expressed by melanosomes	Expressed by normal melanocytes at some stage of differentiation
Tyrosinase	Enzyme that catalyzes the formation of melanin
gp75 or tyrosinase-related protein-1	Transmembrane melanosomal glycoprotein related to tyrosinase; gp gene maps to chromosome 9p23
gp100/pMel-17	

that the significantly prolonged survival of MCV-treated patients reflects inhibition of secondary blood-borne metastasis to vital organs. A phase III trial of MCV is planned. Investigators are currently exploring strategies to augment MCV's immunopotency via new adjuvants and/or gene transfection (Morton DL, ACC95, pp 23-25).

A partially purified, polyvalent, melanoma antigen vaccine from material shed into culture medium by melanoma cells is under clinical evaluation at Kaplan Comprehensive Cancer Center at New York University Medical Center (New York, NY). The vaccine contains a broad range of antigens to ensure that most relevant tumor antigens are present in order to circumvent the antigenic heterogeneity of melanoma and to stimulate multiple antitumor immune effector mechanisms. In clinical trials the vaccine was safe with only one minor complication in almost 400 patients treated for periods of several months to up to 5 years. The vaccine stimulated humoral and cellular immune responses against melanoma, including cellular responses to a patient's own tumor in more than 50% of cases. In combination with liposomal IL-2, used as adjuvant, the vaccine induced strong cellular immune responses in more than 75% of patients. Duration of disease-free and overall survival of treated patients with surgically resected stage III melanoma, was 50% longer than that of controls. Survival was particularly prolonged in patients who developed a strong immune response to the vaccine; such response was independent of disease severity or of the patient's overall immunologic competence. A randomized clinical trial is in progress (Bystryn, Jean-Claude, etal, ACCR95, pp 53-54).

Plasma membranes that contain the entire antigenic repertoire of a cell in the form of processed antigens presented as peptides by major histocompatibility complex (MHC) class I molecules, may also be used as active specific immunization strategy. These membranes were shown to stimulate human melanoma-specific cytotoxic T lymphocyte (CTL) clones *in vitro* in an antigen-specific MHC class I-restricted manner. The ability of these membranes to prime a CTL response *in vivo* suggests that they may be used as T-cell vaccines. This approach circumvents the difficulties associated with generating of human tumor cell lines and identifying CTL-recognized determinants (Heike M, Journal of Immunotherapy with Emphasis on Tumor Immunology, 1994 Apr, 15(3):165-74).

Cell surface gangliosides GM2, GD2, and GD3 are often overexpressed in malignant melanoma. Also, patients with high titer of GM2 antibodies survive longer. Immunization with GM2 and Bacillus Calmette-Guerin (BCG) induces an IgM antibody response in most melanoma patients but, as commonly seen with carbohydrate antigens (which are T independent), the IgM response is short lived and an IgG response is rare. To increase immunogenicity, GM2 was conjugated covalently

with keyhole limpet hemocyanin (KLH) and administered to melanoma patients alone or with one of the three adjuvants, BCG, Detox, or saponin fraction QS-21. The most effective combination, GM2-KLH with QS-21, induced a much higher titer, a longer-lasting IgM GM2 antibody response and a consistent IgG response. It also induced the highest titer anti-KLH response. Because no serious toxicity was observed, this vaccine may be used to augment immunogenicity of other gangliosides, such as GD2 and GD3, and to determine the effects of ganglioside antibodies on the course of melanoma (Helling F, etal, Cancer Research, 1995 Jul 1, 55(13):2783-8). Cambridge Biotech (Worcester, MA) is developing QS-21 as a vaccine adjuvant.

Ribi ImmunoChem Research is currently evaluating, in pivotal phase III human clinical trials in patients with stage II (primary) melanoma, its Melacine melanoma theraccine which incorporates Detox adjuvant with human melanoma tumor cell (mechanical) lysate as the antigen. The tumor cell lines used to manufacture Melacine have been licensed from the University of Southern California (USC; Los Angeles, CA). The ongoing study is being conducted by the Southwest Oncology Group with sponsorship from the National Cancer Institute (Bethesda, MD). Patient accrual is expected to be completed in 1995. Results from a phase III clinical trial completed in 1994 that compared Melacine and combination chemotherapy (DTIC + cisplatin + carmustine + tamoxifen) in 140 patients with stage IV melanoma (each treatment arm included 70 persons, of which 16 in each group were unevaluable for response), showed that median survival for evaluable Melacine and chemotherapy patients was comparable (329 days versus 373 days). However, based on its efficacy and safety profiles, Melacine may be an appropriate alternative to chemotherapy for treating disseminated melanoma. There was a significant quality-of-life effect in favor of Melacine during active treatment. Furthermore, for both adverse events and clinical laboratory parameters, Melacine was safer and better tolerated than chemotherapy. Preliminary evaluation of objective responses demonstrated that two Melacine-treated patients experienced complete responses (CRs), one a partial response (PR), and disease stabilized in five. Those on chemotherapy had two CRs, 11 PRs, and disease stabilized in 23 patients (Mitchell MS, etal, ICC95, Can J Infec Dis, July 1995, Vol 6 Suppl. C; Pg 347C:2027).

Lidak Pharmaceuticals (La Jolla, CA) received FDA clearance in August 1995 to begin a phaseI/II trial of its Large Multivalent Immunogen (LMI) product, LP-2307, as a potential treatment for malignant melanoma. The trial will enroll 18 patients with advanced (stage III and IV) disease, and will last about one year. The study will evaluate the ability of LP-2307 to elicit immunological responses, in particular the induction of CTL. LMIs are created by attaching isolated tumor antigens in high concentrations onto cell-size microspheres which are then injected into the patient. LMI is designed to stimulate the patient's own immune system to attack tumor cells. In experimental animal models LMIs stimulated CTL responses to a variety of tumors and improved survival. The company holds USA (1991) and European (1995) patents for the use of LMI technology in cancer immunotherapy. Lidak acquired the LMI technology from its developer, the non-profit Medical Biology Institute (La Jolla, CA), pursuant to a long-term exclusive worldwide licensing agreement. In August 1993 Lidak entered into a licensing agreement with Ribi ImmunoChem Research under which Ribi supplied Lidak with its proprietary melanoma cell lines. In return Lidak granted Ribi an option for an exclusive license to use of LMI technology with its melanoma cell lines.

Cytel's (San Diego, CA) Theradigm (CY-21100), an antigen-specific immunostimulant, entered phase I/II clinical trial in MAGE-3-associated melanoma in mid-1994 (also see FO, V1 #2/3, p 52). In March 1995 Cytel applied for world patents on a sequence of MAGE-1 antigen for stimulating the immune response to melanomas and on the therapeutic, *in vitro* activation of CTL with peptide loaded presenting cells.

StressGen Biotechnologies (Victoria, BC, Canada) is developing vaccines (Oncocine) which combine tumor antigens with microbial stress-induced (heat shock) proteins (hsp65 and hsp71) using technology (Unigen) exclusively licensed from the Massachusetts Institute of Technology (MIT; Cambridge, MA). Heat-shock proteins (HSPs) continue to appear with intriguing regularity among antigens involved in immune response to cancers (RA Young, A Rev Immun, [1990] 8:401), and are among the most highly conserved proteins in living systems. HSPs offer an alternative to purified antigen preparations by making prior determination of the antigenic epitopes of cancer cells unnecessary (Udono, H and Srivastava PK, J Exp Med, [1993] 178:1391; Srivastava PK, Experientia, [1994] 50:1054). Recent evidence indicates that HSPs gp96, hsp90, and hsp70 associate with antigenic peptides derived from cellular proteins, suggesting that HSPs may not be immunogenic per se, but that they may act as carriers of antigenic peptides. In this scenario, HSPs are released from tumor cells *in vivo* during lysis of the cells by the action of antibodies or nonspecific effectors; the HSPs, which are now complexed with antigenic peptides derived from the cognate cells, are then taken up by macrophages or other specialized antigen-presenting cells, possibly through a receptor-mediated mechanism. The HSP-borne peptide is then routed to the endogenous presentation pathway in the antigen-presenting cell, and is displayed in the context of that cell's MHC class I molecules where it is finally recognized by precursor CTLs (PK Srivastava, etal, Immunogenetics, [1994] 39:93). Indeed, studies have shown that homogeneous preparations of HSPs derived from any cell type contain a wide

assortment of peptides (6-35 mers) noncovalently bound to or "chaperoned" by the HSP (Li Z and Srivastava PK, EMBO J, [1993] 12:3143). One consequence of this phenomenon is that HSP preparations contain the entire repertoire of peptides generated in a cell, a repertoire consisting not only of self peptides but antigenic peptides. Thus, HSPs derived from tumors are complexed with peptides derived from tumor antigens. The vaccination of animals with such HSP-peptide complexes elicits CD8+ T cells specific for the antigenic peptides present in the HSP preparation, resulting in tumor-specific protective immunity (Udono H, etal, Proc Natl Acad Sci USA, [1994] 91:3077); this immunity appears to be general, since three of the major cellular HSPs, hsp70, hsp90 and gp96, have been shown to act as cancer vaccines against three antigenically distinct murine sarcomas (Srivastava PK, Adv Cancer Res, [1993] 62:153; Udono H and Srivastava PK, J Immunol, [1994] 152:5398).

Active Specific Immunotherapy-Gene Transfer

The two technical approaches to genetically modified human cancer vaccines include *ex vivo* gene transfer of cytokine and other genes into tumor cells followed by reimplantation, as well as *in vivo* gene transfer by direct physical application or injection of the gene to transfect the tumor cells and/or the surrounding normal tissues.

Combination of gene transfer technology with new findings about the way the immune system views and deals with tumor cells may produce powerful immunotherapeutics with broad anti-tumor applications. Using a melanoma cell line, investigators demonstrated that tumor cells were not immunogenic, i.e. did not react with killer T cells, unless a surface molecule, B7, was present. When mice were injected with B7-bearing tumor cells, all tumors formed completely regressed within a few weeks. Also, 40% of melanoma tumor-bearing mice injected with cells engineered to produce B7, survived tumor-free.

Several groups are pursuing animal work in this area. Investigators at Bristol-Myers Squibb Pharmaceutical Research Institute (Seattle, WA) have used gene transfer of B7 along with a gene for a viral antigen known to elicit a strong immune response. Researchers at the University of California, Berkeley, obtained similar results without costimulation.

Melanoma cells incorporating the B7 gene may be used as a vaccine. When injected into mice they protected the animals from later challenges by unaltered tumor cells; 89% of the mice were tumor-free for more than 3 months. Tumor cells engineered to produce B7 directly stimulate tumor-specific killer T cells, resulting in increased production of various immunostimulatory cytokines, such as IL-2 (such cytokines are normally produced by helper T cells). Human clinical trials with B7-transduced melanoma cells are being planned.

Various vectors for gene transfer are being evaluated in humans. One such vector, vaccinia, was highly effective in infecting/transfecting normal melanoma cells *in vitro* and *in vivo.* A pilot study involving 5 patients with dermal, subcutaneous and/or lymph node metastasis was conducted to determine safety and if pre-existing or induced immunity to vaccinia restricts the efficacy of sequential administration *in vivo*. Following revaccination vaccinia vaccine was injected intralesionally with no significant adverse side effects and one patient with a large exophytic lesion experienced dramatic tumor regression (Mastrangelo MJ, etal, AACR95, Abs. 2751).

Another approach to introduce genes into melanoma tumors to stimulate anti-melanoma T-cell immunity is by particle-mediated gene transfer (PMGT) being developed by Agracetus (Middleton, WI). In PMGT, melanoma cell lines were successfully transduced with cDNAs for IFN-γ by bombarding them with gold beads coasted with human interferon-γ (IFN-γ) cDNA (Albertini MR, etal, AACR95, Abs 2934).

Transfection with the inducible nitric oxide synthase (iNOS) gene induces apoptosis and suppresses tumorigenicity and metastasis in murine melanoma. Nitric oxide, produced by cells transfected with active iNOS gene, suppressed growth of bystander tumor cells (Xie K, etal, ACC95, pp 71-72).

The most intensively investigated approach to genetically-modified tumor vaccines has been the introduction of cytokine genes. This strategy does not involve inducing the expression of any foreign genes in tumor cells, but seeks to locally alter the immunologic environment of tumor cells to either enhance antigen presentation of tumor-specific antigens to the immune system or enhance the activation of tumor-specific lymphocytes. One of the most important concepts underlying the use of cytokine gene-transduced tumor cells is that very high concentrations of the desirable cytokine is released *in situ*, with systemic concentrations generally being quite low. This paracrine release much more closely mimics the natural biology of cytokine action than does the systemic administration of recombinant cytokines. Many cytokine genes have been introduced into tumor cells with varying effects on both tumorigenicity and immunogenicity. Some of these cytokines, when produced by tumors, induce a local inflammatory response that results in elimination of the injected tumor. This local inflammatory response is often predominately dependent on leukocytes other than classical T cells. In addition to rejecting the genetically-modified tumor cells, vaccinated animals have, in some cases, developed a T cell-dependent systemic immunity which has cured micrometastases established prior to treatment with the genetically-altered tumor cells.

Among models of paracrine cytokine production, granulocyte-macrophage colony-stimulating factor (GM-CSF) was the most effective in inducing systemic immune

responses in poorly immunogenic melanoma cell lines. For instance, Schering-Plough Research Institute (Kenilworth, NJ) investigators discovered that pretreatment of B-16 melanoma cells with GM-CSF enhanced their immunogenicity. After immunization with irradiated cells that had been pretreated with 10-100 ng/ml of CM-CSF, 40% of the mice did not form tumor after subsequent challenge with the same tumor cells compared to only 10% of controls (immunization of athymic mice with this method was ineffective, indicating that the immunity is T-cell mediated). A single vaccination decreased growth of primary tumors by 50% and also decreased the number of lung metastasis by 35%. The induced immunity was tumor specific, since immunization with GM-CSF pretreated B16-F10 cells did not prevent the growth of M27 carcinoma in mice. This immunotherapy approach is now being evaluated in humans (Maxwell E, etal, AACR95, Abs 2922).

Immunization with tumor cells transfected with CM-CSF gene also prevented tumor formation in animals challenged with parental tumor cells. In a similar vein, introduction of the gene for macrophage colony stimulating factor (M-CSF) into murine melanoma cells caused an effective antitumor response, prolonging survival of treated mice over controls (Walsh P, etal, J Natl Cancer Inst 87:809-816, 1995).

Immunex (Seattle, WA) has entered into a collaborative agreement with Johns Hopkins University (Baltimore, MD) to develop controlled-release microsphere formulations of yeast-derived GM-CSF to be used as a vaccine adjuvant.

Somatix Therapy (Alameda, CA) is developing a gene therapy approach, GVAX, in which tumor cells are removed from patients, transduced with the gene for GM-CSF, irradiated, and then reinfused into patients. The company begun a phase I/II dose ranging trial with GVAX in advanced metastatic melanoma patients at Dana-Farber Cancer Institute and Massachusetts General Hospital (Boston, MA) in March 1995. Three groups of three to five patients are to be administered GVAX on a weekly, fortnightly or monthly basis for three months. Study endpoints are safety and generation of an immune response. Differences in potency between subcutaneous and intradermal injection routes will also be established. A 28-patient phase I/II clinical trial of GVAX in advanced metastatic melanoma is also being conducted at the Netherlands Cancer Institute in Amsterdam. Patients received either three vaccinations of five or 50 million cells at 21 day intervals, or control injections of GM-CSF or unmodified irradiated cells. Although cancer progressed in seven patients, it stabilized in three who remained tumor free for up to eight months. In October 1995, Somatix announced that it obtained a worldwide exclusive license from Yale University (New Haven, CT) for orally delivered genes using adeno-associated viral vectors.

Targeted Genetics (Seattle, WA), in collaboration with UCLA Medical Center, began phase I trials of an interleukin-7 (IL-7) vaccine for the treatment of melanoma after RAC approved the protocol in March 1995 (it had been deferred for approval at RAC's June 1994 meeting). As proposed by principal UCLA investigator James Economou, MD/PhD, the study plans to assess the safety and immunostimulatory effects of administering IL-7-producing melanoma cells in patients with metastatic melanoma. This is the first-ever administration of IL-7 in humans. The open-label, phase I clinical trial will test safety and immunologic effects of administering IL-7-producing melanoma cells in nine patients with metastatic melanoma. Under the *ex vivo* treatment protocol, patients will receive irradiated unmodified autologous tumor cells mixed with allogenic melanoma cells transduced with IL-7/HyTK retroviral vector to produce IL-7. The treatment will be given in three biweekly subcutaneous inoculations.

Boehringer Ingelheim, through its Biberach, Germany and Institute of Molecular Pathology (Vienna, Austria) research centers, is evaluating in phaseI/II as of May 1995, a malignant melanoma vaccine (BIWB 1) consisting of the patient's own irradiated tumor cells containing the IL-2 gene. Tumor cells are taken from patients, genetically modified to express IL-2 and, after their ability to divide is inhibited; they are reinjected into patients in order to induce an immune response to the tumor. The technique uses a receptor-mediated adenovirus-augmented gene delivery system (Zatloukal K, etal, Journal of Immunology, 1995, 154: 3406-3419). The first patients were recruited for a clinical trial in the last quarter of 1994. In 1994 a pilot plant was built in Vienna for GMP-standard production of tumor vaccines following approval by the Austrian authorities.

RGene Therapeutics' (The Woodlands, TX) cationic lipid vector, a non-viral vector, was successfully used by academic researchers at the University of Michigan (Ann Arbor, MI) for the treatment of melanoma and is being developed by Genzyme (Cambridge, MA) for the treatment of cystic fibrosis. Aronex (was Argonex/Argus; The Woodlands, TX) is producing the liposomes under an agreement with RGene.

Genetic Therapy (Gaithersburg, MD), a unit of Sandoz acquired in July 1995, is clinically evaluating, under investigator INDs, *ex vivo* gene transfer of TNF and IL-2 (in collaboration with NIH), IL-4 (University of Pittsburgh) and various markers for the treatment of malignant melanoma. The company is also collaborating with Sandoz Pharma to develop and market gene therapy products based on its simplex virus-thymidine kinase (HS-tk) technology for the treatment of solid tumors. The company is collaborating closely with W. French Anderson, director of USC's Norris Cancer Center (Los Angeles, CA), in developing gene-based therapies for cancer.

Vical (San Diego, CA) is clinically evaluating Allovectin-7 which contains a gene that encodes a mismatched transplantation antigen (HLA-B7) which, when intralesionally injected, is intended to cause malignant cells to bear the foreign antigen on their surface. Vical started a phase I/II clinical trial of Allovectin-7 in the treatment of malignant melanoma in late 1994 to test the safety of Allovectin-7 at varying dosage levels and to assess HLA-B7 gene transfer and expression in tumor lesions following administration. During the 1995 AACR meeting Vical and scientists from the University of Arizona (Tucson, AZ) reported on the biochemistry and immunology laboratory results obtained from administering Allovectin-7 to 40 patients with advanced cancers, including metastatic melanoma, colorectal carcinoma and renal carcinoma (Harris D, etal, AACR95, Abs 1312). Data presented showed that:

- direct gene transfer into different tumor types appeared to be safe and feasible
- the HLA-B7 gene and protein were transferred and could be detected for several weeks in greater than 75% of the patients (28 of 36 available biopsies)
- intratumoral T cell infiltration (10 of 11 tested) and a functional cell mediated immune response was observed (3 of 8 tested) in patient samples

Measurable tumor shrinkage was observed in 50% of 14 patients with advanced melanoma, including three partial remissions. Vical began a phase II efficacy trial of about 100 patients with various solid tumors to be enrolled at ten sites in the USA and Canada.

Therion Biologics (Cambridge, MA) is developing therapeutics for melanoma by inserting immunomodulators into the company's recombinant pox virus vectors. The company has ongoing collaborative agreements with NCI to develop immunotherapeutic vaccines combining antigens identified at NCI or other selected immunomodulators such as cytokines. The company has isolated antigens to incorporate in its vaccines through technology developed by Steven Rosenberg, MD, at the NCI. This technology uses tumor infiltrating lymphocytes (TILs), which possess the ability to recognize antigens specific to malignant cells. The first clinical trials to result from the NCI collaboration involve the MAGEVAC vaccine engineered to express the MAGE-1 peptide believed to be produced by approximately 33% of melanomas and 20% of breast tumors. In a phase I trial of subjects with metastatic melanoma expressing the MAGE-1 antigen, the vaccine produced no adverse reactions in recipients other than the normal swelling associated with smallpox vaccine injection. Future Therion immunotherapeutic vaccines will incorporate MART-1 and gp100 antigens first identified using TIL technology, that are found on nearly 100% of melanoma cells. Therion and Therion is collaborating with the NCI through a Collaborative Research and Development Agreement (CRADA), under which Therion has a first option for commercialization rights for the use of MART-1 and gp 100 in poxviruses. The company is also exploring the use of co-stimulatory molecules, such as cytokines, in combination with MART-1 and gp 100.

Active Specific Immunotherapy-Monoclonal Antibodies

A novel "molecular" approach to the construction of tumor vaccines is based on the concept of idiotypic mimicry using anti-idiotypic (anti-Id) monoclonal antibodies (MAbs). The region of an antibody which is unique to that antibody, termed the idiotope, contains the antigen-combining site, although it is not identical to it. Ids may act as antigenic determinants and induce the formation of antibodies; these anti-Ids may be directed against determinants directly involved in antigen-antibody interaction within the antigen binding site or determinants that define the structure of the variable region. Anti-Ids may mimic the structure of the epitope of the original antigen, and are thus said to bear an "internal image" of the antigen. Thus, it may be possible to use anti-Id vaccines in ASI as a substitute for specific antigens derived from the tumor cells themselves, inducing antibody reactions against tumor antigens which have not otherwise been immunogenic (PB Chapman and AN Houghton, in Biologic Therapy of Cancer, DeVita Jr VT, et al, eds., JB Lippincott, New York, 1992, p 1).

A murine anti-Id, MAb MK2-23, that bears the internal image of the antigenic determinant defined by the anti-human high-molecular-weight melanoma-associated antigen MAb 763.74, was clinically evaluated by investigators at New York Medical College (Valhalla, NY) in patients with advanced malignant melanoma. The immunogenicity of MAb MK2-23 was enhanced significantly by conjugation to the carrier keyhole limpet hemocyanin (KLH) and by co-administration of BCG. MAb MK2-23 induced humoral immunity in about 60% of immunized patients (Ferrone S and Mittelman A, AAC95, pp 56-57).

ImClone Systems (New York, NY) is clinically evaluating BEC2 (LuVax/MelVax), a mouse anti-Id that mimics the ganglioside antigen GD3. Phase Ib/IIa clinical studies of BEC2 in patients with melanoma and small cell lung carcinoma are underway.

Adoptive Immunotherapy

An alternative approach to the active immunotherapy of cancer involves the transfer of immune cells designed to react against the cancer. In adoptive (or cellular) immunotherapy cells with anticancer reactivity that can mediate either directly or indirectly anticancer effects on growing tumor are transfered to the tumor-bearing host. Research in cellular immunology showed that it was the cellular rather than the humoral arm of the immune response that was involved in tissue and organ allograft rejection and in the rejection of experimental tumors. In

these studies, the immunity to tissues present in highly immunized mice could be transferred to virgin mice by the adoptive transfer of lymphocytes, whereas the transfer of serum containing large amounts of antibody was relatively ineffective in transferring immunity. In general, animals treated by adoptive immunotherapy manifested systemic immunity by their ability to reject subsequent tumor challenges in an immunologically specific manner. These findings led to efforts to transfer lymphocytes among cross-immunized cancer patients. However, these efforts were largely unsuccessful due to the limited ability to immunize patients against allogeneic cancer-associated antigens *in vivo* and the limited survival of allogeneic lymphocytes in the host. The major obstacle to the development of effective adoptive cellular therapies for the treatment of cancer in humans was the inability to generate cells from cancer-bearing patients that had specific immune reactivity against cancer and that could be generated in large enough numbers. Several approaches to raising immune cells designed to overcome these obstacles have been developed, and have led to the first examples of successful cellular therapy of advanced cancer in humans. These studies have included the use of LAK cells, TIL, gene-modified cells, and tumor-sensitized lymph node cells.

Cellcor (Newton, MA) has treated 36 patients with disseminated melanoma using autolymphocyte therapy (ALT) alone (26 patients) or adoptive chemoimmunotherapy using ALT and cyclophosphamide (10 patients). ALT involves infusion of autologous PBMCs activated *ex vivo* by a cytokine-rich supernatant (T3CS) harvested from a previous autologous lymphocyte culture using low doses of the mitogenic MAb anti-CD3. The mechanism of action is enhancement of a recall response memory T cells (ALT-cells) to host tumor without dependence on exogenous IL-2. A total of 161 infusions of ALT-cells were given (96 with T3CS) and 61% of patients received the planned 6 ALT-cell infusions. In 33 evaluable patients, there were 4 CRs, 4 PRs, and 6 patients with SD (Osband, ME, etal, ASCO95, Abs. 1328). In June 1995, Cellcor signed a definitive agreement to be acquired by Cytogen (Princeton, NJ).

Passive Immunotherapy

Binding of antibodies to antigens on the surface of tumor cells is generally necessary, but not sufficient, to inhibit tumor growth. Antibodies with appropriate isotypes can trigger complement-dependent cytotoxicity or participate in antibody-dependent cell-mediated cytotoxicity (ADCC) after binding to antigen. However, many human tumor cells are relatively resistant to lysis by human complement components and effectors for ADCC may not be plentiful within the tumor compartment or their function may be impaired by several factors including the presence of endogenous antigen-antibody complexes. In addition to the potency (or lack thereof) of effector mechanisms, several other factors may impede the efficacy of MAbs, including complexing with shed antigen, failure to penetrate tissue, antigenic modulation, heterogeneity of antigen expression, and the development of human anti-mouse antibodies (HAMA). Fortunately, not all cell surface antigens are shed and not all shed antigens impede reactivity of MAbs with tumor cells. And while saturation of antigenic sites on solid tumor nodules has proven difficult to achieve, a significant fraction of sites can be occupied by MAbs after intravenous injection. Of course, even when antibody can gain tumor access, a fraction of tumor cells may fail to express any given antigen; however, the use of multiple antibodies in combination can compensate for this heterogeneity. Despite the many potential limitations of passive immunotherapy, if experience in animals holds true, it is possible that dispersed tumors will respond to therapy with passively transferred antibodies. Such dispersed tumors are accessible to antibodies that leave the circulation and to antibody-armed macrophages and NK cells.

Gene Transfer-HSV Thymidine Kinase Gene

The ability of tumor-infiltrating lymphocytes (TILs) to specifically accumulate at tumor sites has suggested the possibility that TILs might be used as vehicles to deliver molecules to the tumor with improved antitumor activity. At the same time, however, many of the target antigens being identified in human tumors for immunologic attack are also found on normal tissues, and amplifying T cell responses specific for such proteins by infusions of large numbers of reactive T cells could result in injury to normal tissue. Thus, researchers are examining methods to introduce genes into T cell clones that could potentially both improve the safety of adoptive T cell therapy and enhance the efficacy of the transferred T cells.

At the University of Washington and Fred Hutchinson Cancer Research Center (Seattle, WA), Philip D. Greenberg's group is utilizing a retroviral vector to introduce the herpesvirus thymidine kinase (HS-tk) gene as an inducible "suicide" gene into T cells prior to transfer to permit the ablation of transferred cells by the administration of ganciclovir if toxicity develops (Lupton SD, et al, Mol Cell Biol, [1991] 11:3374; Greenberg PD, et al, in 1994 Vaccines Against Virally Induced Cancers, Ciba Foundation Symposium 187, Wiley, Chichester, 1994 p 212). Gene transfer (using either retrovirus or adenovirus vectors) of Hsv-tk gene into tumor cells renders them sensitive to the cytocidal effect of the antiviral drug ganciclovir. Preliminary studies have demonstrated that T cell clones transduced with this vector and expressing a fusion gene (HyTK) encoding hygromycin phosphotransferase fused in frame to HS-tk, can be ablated in tissue culture following exposure to less than 1 μg/ml of ganciclovir; a substantially higher serum level of ganciclovir is readily achievable in patients without toxicity. Preliminary studies in mice have also demonstrated that T cell clones expressing the HyTK gene can be ablated *in vivo* after administration of ganciclovir. Effectiveness of Hsv-tk gene therapy has been attributed to the bystander tumoricidal

effect which was hypothesized to explain tumor eradication in spite of the fact that the efficacy of *in vivo* gene transfer to tumor cells was less than 100%. The bystander tumoricidal effect was shown to be a major contributor to the ganciclovir's cytotoxicity *in vitro*. A maximal tumoricidal effect was seen when only one in 10 tumor cells expressed the Hsv-tk gene which suggests that in effect, one tumor cell with the Hsv-tk gene, when given ganciclovir, destroys 10 neighboring or bystander cells. The destruction of bystander cells does not appear to be mediated by a soluble factor(s) released into the media but, rather, requires close cell proximity or cell contact (Wu JK, etal, Neurosurgery, 1994 Dec, 35(6):1094-102; discussion 1102-3). Similar results were obtained with an *in vivo*, adenovirus-mediated gene therapy approach for the treatment of malignant melanoma (Bonnekoh B, etal, Journal of Investigative Dermatology, 1995 Mar, 104(3):313-7).

Pasteur Institute (Paris, France) researchers used a direct virus-free transfer of a plasmid (pAG0) for transduction of Hsv-tk gene in mice bearing melanoma tumors. Pathological analysis of melanoma tumors from mice treated with this approach showed 75% of necrosis which was not seen in controls (Soubrane CL, etal, AACR95 1309).

Genopoietic, a privately held French company, was established in April 1993 as the exclusive licensee of gene therapy technologies patented by the Pierre and Marie Curie University (Paris, France). It has a product based on HS-tk anticancer technology in Phase I/II trials in melanoma, and has received approval to start phase I/II trials in glioblastoma. RPR Gencell (Rhône-Poulenc Rorer) has exclusive worldwide rights to all HS-tk antitumor programs developed by Genopoietic, and right of first refusal on other programs.

OTHER AGENTS IN DEVELOPMENT

Dovetail Technologies

Dovetail Technologies (DTI; Silver Spring, MD), founded in 1990, is focusing on small thiol compounds (Betathines), based on vitaltheine, recently identified as having strong antitumor and immunomodulatory properties. In animal studies, Betathine-2 was found to be potent, showed evidence of B-cell differentiation and modulation of *in vivo* T-cell response (as measured by delayed-type hypersensitivity testing) and a 70% apparent cure in a relatively late intervention melanoma model without toxicity even at relatively high doses (no acute lethal responses at a 250 mg/kg dose, which is more than two orders of magnitude higher than the effective dose). Untreated mice died within 15 weeks while 80% of the optimally treated animals were alive at 16 weeks and nearly all the surviving mice (88%) were apparently completely cured. DTI has the exclusive worldwide license for the use of these compounds. European Patent Office and USA patents have been allowed or issued. To have high-grade compounds for testing in animal and human trials, DTI established a corporate partnership with Hauser Chemical Research (HCR; Boulder, CO). HCR is an investor in DTI and is producing Betathines under conditions suitable for FDA approval. Hauser will provide chemistry and manufacturing services for Dovetail's compounds, in exchange for equity in Dovetail.

Melatonin

The pineal hormone melatonin may be effective as adjuvant therapy in node-relapsed melanoma patients. In addition to its immunomodulating activity, melatonin has been shown to inhibit a number of cancer cell lines including melanoma cells. A study was set up to evaluate the impact of melatonin adjuvant therapy on progression-free survival of node-relapsed melanoma patients. Thirty persons were randomized after radical node excision to receive no drug or melatonin 40 mg daily orally in the evening every day until progression. At median follow-up of 18 months, the percentage of recurrence was significantly lower in patients treated with melatonin versus those who received no adjuvant therapy. Also, no melatonin toxicity was observed (Brivio F, etal, ICC95, Can J Infec Dis. July 1995, Vol 5 Suppl C; Pg 347C:2029).

Melanoma Inhibitory Activity (MIA) Protein

Melanoma inhibitory activity (MIA) protein, a novel malignant melanoma-derived growth regulatory protein that was recently purified and cloned, may have a potential therapeutic use as an anticancer drug. MIA was expressed in 100% of malignant melanoma cell lines but not in normal melanocytes. Growth inhibition (up to 90%) by recombinant human MIA was mainly observed in tumors with detectable message. Normal fibroblasts, the majority of non-melanoma cells and lymphocytes were not growth arrested (Blesch A, etal, AACR95. Abs. 260). Recombinant MIA inhibits both the proliferation of melanoma cells and also their invasive potential *in vitro* by specifically inhibiting attachment by modulating cell-matrix interactions. MIA binds to RGD-peptides and RGD-containing proteins. Thus, it is likely that MIA regulates cell-matrix interaction by a novel mechanism, i.e. interfering with the interaction between integrins and RGD-sequences of the extracellular matrix (Bosserhoff Anja-Katrin, etal, AACR95, Abs. 433).

SunPharm

SunPharm (Jacksonville, FL) is developing diethylnorspermine (DENSPM), which entered phase I clinical trials in January 1994 at Johns Hopkins Oncology Center (Baltimore, MD), the University of Florida (Gainesville, FL) and Roswell Park Cancer Institute (Buffalo, NY) in a variety of solid tumors (see FO, V1 #4, pp 102 & 108).

Cancer Research Campaign Technology

Cancer Research Campaign Technology (London, UK) is developing temozolomide, an imidazotetrazine derivative (licensed to Schering-Plough), for the treat-

Exhibit 3
Drugs in Development for the Treatment of Melanoma

Primary Developer/ Affiliate(s)	Generic Name/ Number/Brand Name	Drug Type/Target/ Mechanism/Delivery	Status/Location/ Indication	Comments
Ajinomoto/Nagasaki U, BioChem Pharma	Recombinant BCG (Bacillus Calmette Guerin) vaccine	Host-vector system applied to secretion of foreign antigens from living BCG using the α-antigen as a carrier/intra and extra cellular antigens	Clinical/Japan	Approved/USA, Canada/bladder cancer
AmpliMed (licensee)/ Research Corporation Technologies (licensor)/ U Arizona, Arizona Cancer Center (original developer)	Azonafide compound	Anthracene analogs of amonafide/topoisomerase inhibitor	Preclin/USA/ B16 melanoma	
Andrulis	NSC-608913/Polyplat	Platinum-based/Pt (trans-1,2-diaminocyclohexane) with carboxyamylose	Preclin/USA	
Anutech	γ-inulin	Activator of the alternative pathway of complement (APC)	Phase I/Australia	
Asahi Chemical/ FZB Biotechnik	rTNF-α, cachectin, PAC-4D	Growth factor/IV	Phase I/Japan (in combination with melphalan)	
Boehringer Ingelheim (Viennese Institute of Molecular Pathology)	BIWB	Receptor-mediated, adenovirus gene delivery system/ gene expressing IL-2 inserted into tumor cells/*ex vivo*	Phase I (11/94)/Europe/ metastatic melanoma	
Braton Biotech/NCI (NIH), CRC Dept of Medical Oncology	Brefeldin A/BFA, NSC-89671	Macrolide derived from *Penicillium brefeldianum*/ inhibits movement of membrane proteins from the endoplasmic reticulum to the Golgi/intra-cellular trafficking/IV	Preclin/USA, UK	.
Bristol-Myers Squibb	BMS-181174; BMY-25067	Mitomycin-C analog/IP	Phase I/The Netherlands, UK	
Bristol-Myers Squibb	BMS-182248-01; BR96-DOX	Immunoconjugate/chimeric (mouse-human) IgG_1 anti-$Lewis^y$ MAb conjugated to doxorubicin/RNA synthesis inhibitor/targets epithelial cancer	Phase I/USA	
Bristol-Myers Squibb	Etopophos; etopofos/ BMY-40481	Water-soluble analog	Phase II/USA	See FO, V1 #4, pp 95 & 103
British Technology Group (BTG)	C-1311	Substituted imidazoacridinone	Preclin/UK/ B16 melanoma	Does not generate reactive oxygen species implicated in anthracycline-type cardiotoxicity
British Technology Group (BTG)	AQ4N	Radiosensitizer/enhances drug cytotoxicity against hypoxic tumor cells	Preclin/UK/melanoma, solid tumors	
British Technology Group (BTG)	Viral hemagglutinin-neuraminidase (HN)	Membrane glucoprotein/ promotes intra cell interactions; improves efficacy of tumor vaccines	Preclin/UK	
Cambridge Biotech/ Memorial Sloan-Kettering Cancer Institute	Granulocyte-macrophage-2-keyhole limpet hemaocyanin vaccine (GM2-KLH) + saponin	Vaccine	Phase II/USA	Adverse effects included mild local tenderness, erythema and induration at vaccination sites lasting fraction QS-21 adjuvant 2-4 days
Cancer Research Campaign (CRC) Technology/Schering-Plough (licensee); May & Baker	Temozolomide/ CCRG-81045; M&B-39831; NSC-362856; RP-46161; Sch-52365	Oral alkylating agent; imidazotetrazine derivative/ PO	Phase II/III/UK	Rhône-Poulenc Rorer discontinued development

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Cancer Research Campaign	CB10-277	DTIC analog with improved solubility, stability and (possibly) metabolic activation/IV	Phase II/UK	Did not exhibit major activity when administered as a slow infusion of 12,000 mg/m^2 over 24 h q 3 weeks (Bleehen NM, etal, British Journal of Cancer, 1994 Oct, 70(4):775-7)
Cellcor (Cytogen)	Autolymphocyte therapy (ALT)	*Ex vivo* cancer therapy	Clinical/USA/ melanoma	Phase III/USA/metastatic renal cell carcinoma
Chiron	Anti-bFGF, anti-FGF/11A8-SAP	Anti-basic fibroblast growth factor/anti-FGF receptor, 11A8, linked to saporin	Discontinued	
Ciba-Geigy	CGP-35269	Recombinant hybrid IFN-α		
Ciba-Geigy	Methylglyoxal bis guanylhydrazone (MGBG)/ GGP 48664A	Polyamine biosynthesis inhibitor/potent inhibitor of S-adenosylmethionine decarboxylase/antiproliferative	Research/USA	
Ciba-Geigy/ Chiron Biocine	CGP-19835A, CGP-19835, L-MTP-PE, MF-59, MLV-19835A, MTP-PE	Liposome-encapsulated muramyl tripeptide phosphatidylethanolamine (L-MTP-PE)/ potent activator of monocytes and macrophages/IV	Phase II/USA	In a double-blind placebo-controlled trial in dogs median disease free interval was 346 days compared to 191 days in the placebo group (AACR95, Abs. 2940)
Ciba-Geigy/ QLT PhotoTherapeutics (was Quadra Logic Technologies; ww licensee)	Zinc phthalocyanine/ CGP-55847	Photosensitizer/formulation of zinc II-phthalocyanine in small liposomes	Preclin/Europe	
Cytel/Sequel Therapeutics (joint venture with Scripps Research Institute aquired by Cytel in 1995)	Theradigm-melanoma/CY-2010	Antigen-specific (MAGE-3) immunostimulant/activates CTLs	Phase II (12/94) under physician IND/USA/ MAGE-3 associated melanoma, less advanced melanoma	See FO, V1, #2/3, pp 52 & 58
Debiopharm/Roger Bellon (Rhône-Poulenc Rorer); Sanofi	Oxaliplatin/1670RB; RP 54780/L-OHP	Platinum-based drug/trans ammine (cyclohexylamine) dichloro dihydroxo platinum; water soluble/IV	Prereg/France; phase II/ Portugal/advanced malignant melanoma	Dose-limiting are GI symptoms and neurotoxicity
Dovetail Technologies/ U New Mexico (Health Sciences Center)	Betathines	Small thiols (sulfur containing compounds)/stimulate B cell differentiation and T cell immunity/immunostimulant	Preclin/USA	Hauser Chemical Research, a corporate partner is producing Betathines for Dovetail
Elan/NCI (NIH)	Phenylacetate, NaPA, NaPB, phenylbutyrate, sodium phenylacetate, sodium phenylbutyrate	Metabolites of phenylalanine; aromatic fatty acids/ differentiation inducers/ suppress growth of neuroectodermal tumors	Preclin/USA	Treatment of melanoma cells in advanced stage of maturation resulted in terminal differentiation (Liu L, Journal of Investigative Dermatology, 1994 Sep, 103(3):335-40)
Fuji Photo Film/Dana-Farber Cancer Institute	FJ-776	Rhodacyanine dyes/IP	Phase I/USA, Japan	
Fujisawa	Rubratin; N-CWS	Natural product isolated from cell wall skeleton of *Nocardia rubra*/macrophage stimulant and killer T-lymphocyte activator, induces IFN-γ	Prereg/Japan	Asta Medica (licensor) suspended development in 1993
GeneMedicine/Corange		Gene transfer by nonviral vectors/expression of cytokines in cancer cells/ *in vivo*	Research/USA	
Genetics Institute/ U Colorado	M-CSF	Gene transfer/modification of tumor cells to release M-CSF	Preclin/USA	

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Genetics Institute/ Wyeth-Ayerst (ww except Japan; Yamanouchi in Japan)	Recombinant interleukin-12/ rhIL-12	Enhances the immune system's killing ability and may trigger production of other immune system regulatory proteins that may initiate an adaptive immune response	Phase I/II (suspended 5/95 due to deaths)/USA	In preclinical models of a variety of cancers, rhIL-12 either caused tumors to shrink or entirely eliminated them (also see FO, V1, #2/3 p 52)
Genetic Therapy (Sandoz)/ NIH, U Cincinnati		Gene transfer by retrovirus Hsv-tk suicide gene/*in vivo*	Preclin/USA	PhaseI/II (completed 1994)/USA/brain cancer (RAC #9303-037)
Genetic Therapy (Sandoz)/NIH	RAC #9008-003	Gene transfer by retrovirus/ TNF/*ex vivo*	Phase I/II (investigator sponsored)/USA	
Genetic Therapy (Sandoz)/NIH	RAC #9007-003	Gene transfer by retrovirus/ IL-2/*ex vivo*	Phase I/II (investigator sponsored)/USA	
Genetic Therapy (Sandoz)/U Pittsburgh	RAC #9209-003	Gene transfer by retrovirus/ IL-4/*ex vivo*	Phase I/II (investigator-sponsored)/USA	
Genopoietic (RPR Gencell has option)		Gene transfer by retrovirus of Hsv-tk suicide gene	Phase II/Europe	
IDEC Pharmaceuticals	Melimmune-1 or Melimmune-2	Murine anti-idiotypic MAb plus adjuvant SAP/mimics separate epitopes on the high molecular weight melanoma-associated proteoglycan	Phase I (suspended)/USA	Enhances cell mediated mmune responses (proliferative and cytotoxic) in melanoma patients by specific and nonspecific mechanisms (AACR95, Abs. 2923)
Ilex Oncology	Eflornithine	Enzyme-activated irreversible inhibitor of ornithine decarboxylase	Preclin/USA/prevention of skin cancers	Originally developed by Marion Merrell Dow
ImClone Systems/ Memorial Sloan-Kettering Cancer Center; Merck KGaA (licensee)	Melanoma vaccine/ BEC-2; EMD-60205/ LuVax; MelVax	Murine anti-idiotypic antibody/mimics ganglioside D3 (GD3)/ immunostimulant	Phase Ib/IIa (94)/USA	GD3 glycolipid on surface of melanoma cells are mimicked and can elicit an immune response; humoral response against GD3 in cancer patient are evoked
ImmunoTherapeutics	ImmTher	Immunomodulator/lipophilic disaccharide peptides related to muramyl dipeptide/ liposome encapsulated	Phase II/USA	Also see FO, V1, #2/3, p 53
ImmunoTherapeutics	Theramide	Vaccine/induces cellular immunity	Phase I/USA/ solid tumors	Long-acting analog of ImmTher
Imutec	Virulizin	Immunomodulator, bovine reticuloendothelium derivative/TNF-α agonist; also promotes release of IL-1β, IL-2 and GM-CSF	Phase II (completed in 1993)/Mexico	Postponed clinical trials in 8/95 until the development of a reliable potency assay to elucidate the agent's mechanism of action
InflaZyme Pharmaceuticals	Fitofosine/IZP-94004	Synthetic phospholipid analog/inhibits signal transduction pathways involved in cancer cell growth and survival	Preclin/Canada	Received a USA patent (No 5,369,097) in late 1994; in 1995 Inflazyme signed a Letter of Intent with BioChem Therapeutic, a subsidiary of BioChem Pharma, for the licensing and development of Fitofosine but, in October 1995, the companies agreed not to proceed with the proposed agreement
International Gene Group (IGG International)	NCCG	Naturally-occurring complex carbohydrate derived from pectin/inhibits metastases	Preclin/USA	Acts as an anti-adhesive by binding with certain lectins (proteins possessing high affinity for carbohydrates) on cancer cells, preventing individual cells from metastasizing

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Lidak Pharmaceuticals/ Medical Biology Institute (licensor)	Large Multivalent Immunogen (LMI)/ LP-2307	Immunostimulant/ induces CTLs	Phase I/II (10/95)	
Lipha (Merck KGaA)/ Cancer Research Campaign	Mitoflaxone/LM-975; NSC-347512	Flavone-8-acetic acid/ induces cytokine production and natural killer (NK) cell activity/IV	Phase I/II/Germany; phase I/USA (in combination with IL-2); discontinued as of 9/95	Combination with IL-2 was very toxic and not effective (Holmund JT, etal, Journal NCI, 1995 Jan 18, 87(2):134-6)
Lithuanian Oncology Centre	Faranox	Derivative of chloroethy-laminophenylacetic acid	Phase I/II/Lithuania/ primary cutaneous melanoma, generalized melanoma	In combination with DTIC and vincristine PR was seen in 50% of cases; in combination with DTIC, vincristine, and aranoza) in 56%
Magainin Pharmaceuticals/Sandoz	Magainins	Natural product; antibiotic	Preclin/USA	
Matrix Pharmaceutical	IntraDose-CDDP	Cisplatin intratumoral delivery as an injectable gel	Phase III/USA	
Medac/Immunological Laboratory	Tumor vaccines	Autologous tumor vaccine	Phase I/Germany	
Meiji Seika Kaisha, Yokohama	Sodium D-glucaro-delta-lactam (ND2001)	Non-cytocidal/directly changes some property of tumor cells	Preclin/Japan	Inhibited spontaneous pulmonary metastases of the highly metastatic B16 melanoma variant at maximal inhibition rate of 99.5%; 6 of 7 animals remained metastasis-free (Tsuruoka T, etal, Japanese Journal of Cancer Research, 1995 Jan, 86(1):41-7)
Menarini	Distamycin analogs/ MEN-10718, -10400, -10558, -10705, -10706,-10716	Phenyl mustard distamycin analogs	Preclin/Italy	In murine models the compounds were more efficacious than melphalan
NCI (NIH)/Sphinx Pharmaceuticals (Lilly)	Urocoumarin-sulfonamide/NSC-646957, - 646958, -646959, -646960	Protein kinase C inhibitor	Suspended	
Parke-Davis (Warner-Lambert)/ DuPont Merck (licensee)	Teloxantrone HCl, moxantrazole/ CI-937; DuP-937, NSC-355644; PD-113309	Anthrapyrazole derivative/ potent topoisomerase II inhibitor at the molecular and cellular level/IV, IP	Phase II/Canada, USA	Closely related to mitoxantrone; unlikely to produce free radicals; may be useful in the same indications as mitox-antrone, especially for patients with cardiac risks, pediatric patients, and those treated with intensified protocols (Leteurtre F, etal, Journal NCI, 1994 Aug 17, 86(16):1239-44)
PharmaMar/EORTC	ET 743	Natural product, marine origin	Preclin/Spain	
Prizm Pharmaceuticals	rbFGF-SAP/ Melo-Stat	Chimeric protein; mitotoxin conjugate of FGF and saponin/ binds specifically to the FGF receptor and inhibits protein synthesis in FGF receptor expressing tumor cells	Preclin/USA	Abolished cell growth in a dose and time depen-dent manner; killing efficiency was significantly greater than that of native saponin alone (AACR95, Abs 2530); combination suramin and bFGF-SAP regimen was more effective (Davol, P, etal, AACR95, Abs. 3817)

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Pro-Neuron/M. D. Anderson Cancer Center (U Texas)	Anthracyclines (doxorubicin analogs designed to be activated by tissue hydrolases)/DNC4 and DNC5	Melanoma-selective	Preclin/USA	Compounds retained full potency against resistant cells (Farquhar D, etal. AACR95, Abs. 2358)
QLT PhotoTherapeutics/ U British Columbia; American Cyanamid; Baylor Research Institute	Benzoporphyrin derivative/BPD; BPD-MAb/Chlorin	Photodynamic therapy	Preclin/Canada	May have applications in skin cancer, including melanoma
Repligen	Recombinant platelet factor (PF) 4	Chemokine protein	Phase I/II (completed)/USA	
Ribi ImmunoChem Research (exclusive rights)/ U Southern California Cancer Center (licensor)/ Biomira (licensee)	Theraccine/Melacine	Melanoma vaccine/ active specific immunotherapy	Phase III (orphan drug)/ USA, Canada/malignant melanoma; phase III/ USA/prevention of tumor recurrence following surgical removal	Trials failed to show efficacy (8/95); is being evaluated in combination with IFN-α 2b
R. W. Johnson Pharmaceutical Research Institute (Johnson & Johnson)	Loxoribine/ RWJ-21757	Immunostimulatory ribonucleoside, 7-allyl-8-oxoguanosine/ exhibits adjuvant activity for B cells, activates natural killer (NK) cells, and enhances the activation of lymphokine-activated killer cells by IL-2	Phase II/USA	No major toxicity resulted with dosage of 5 mg/kg
Sanofi Winthrop	Crisnatol/ 770U82; BW-770; BW-770U82; BW-A770U	Intercalating anticancer agent/topoisomerase II inhibitor/DNA polymerase	Phase II/UK	Wellcome discontinued development
Schering-Plough Research Institute		Immunization with GM-CSF cDNA-transduced tumor cells/tumor specific immunization	Phase I/USA	
Servier	Fotemustine/S-10036/ Muphoran	Nitrosourea	L/France (89), New Zealand (91), Australia (93); reg/Portugal	Crosses the blood brain barrier
Sheffield Medical/ Harvard Medical School	Clotrimazole/ SCHAL-1	Antifungal/inhibits proliferation of tumor cells *in vitro* and in animal models/ depletes cellular stores of calcium and blocks the movement of calcium and potassium across cell membranes/topical	Preclin/USA	Phase I/II (physician-sponsored)/ USA/Kaposi's sarcoma
Sheffield Medical/ Harvard Medical School	Clotrimazole/ SCHAL-3	As above	Phase I/II (physician - sponsored)/ USA/dysplastic nevi	
Somatix Therapy	GVAX	*Ex vivo* transfection of a patient's (autologous) tumor cells with the gene encoding GM-CSF	Phase I/USA	
Southern Research Institute/NCI (NIH); CRC Technology (licensee)	Clomesone/ NSC-338947; SRI-6155	Alkylating anticancer agent, DNA antagonist/ chloroethylation of DNA and cross linking of the DNA double helix	Phase I/USA/ B16 melanoma	
Sphinx (Eli Lilly)	Naphthoqumones/ SPC103751	Protein kinase inhibitors/ preferentially inhibit human melanoma cell growth	Preclin/USA	Inhibited growth of primary melanocytes and metastatic melanoma cells; induced a cytotoxic response with little effect on melanocytes (Yamanishil DT, AACR95, Abs. 2350)

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StressGen Biotechnologies	Oncocine	Combinations of tumor antigens with microbial stress proteins	Preclin/USA	Use StressGen's Unigen technology
Sugen	Platelet derived growth factor (PDGF)/SU101	PDGF receptor antagonist; receptor tyrosine kinase (RTK)	Preclin/USA	
SunPharm/Warner-Lambert (worldwide rights); Nippon Kayaku (licensee); Johns Hopkins U; Roswell Park Cancer Institute; U Florida	Diethylnorspermine (DENSPM)	Synthetic polyamine analog/ inhibits natural polyamine synthesis and depleting cell of existing polyamines causing tumor cells to die	Phase I/USA	See FO, V1 #4, pp 102 & 108
Targeted Genetics	IL-7	Gene transfer/genetically-modified tumor cells to produce IL-7 *ex vivo*/ CTL-based immunotherapy	Phase I/USA	
Therion Biologics/ NIH (CRADA)	MAGEVAX	Gene transfer/ gene encoding MAGE-1	Phase I/USA	
Therion Biologics/ NIH (CRADA)		Gene transfer/gene encoding MART-1 and gp100	Preclin/USA	
Viagene (Chiron)	Gene transfer of IFN-γ	Gene therapy using IFN-γ and IL-2/*ex vivo*	Phase I (8/93)/USA/ advanced melanoma	
Viagene (Chiron)	Gene transfer of IFN-γ	Gene therapy using IFN-γ and systemic IL-2/*ex vivo*	Phase I (5/94)/USA/ advanced melanoma	
Vical	Allovectin-7	Gene therapy/encodes a foreign tissue antigen (HLA-B7), causing tumor cells to bear the foreign antigen on their surface/ injectable	Phase I/II (10/94-6/95); phase II (9/95)/USA and Canada	
Vical	Leuvectin	Gene therapy/IL-2 gene in a plasmid-lipid complex/ intratumoral injection	Phase I/II (4/95)/USA/ solid tumors	
Xoma	Recombinant gelonin immunofusion	Ribosome-inactivating enzyme used in targeted immunofusion	Research/USA	
Zambon	N-acetylcysteine (NAC)	Thiol/affects the process of tumor-cell invasion and metastasis, probably due to inhibition of gelatinases by its sulfhydryl group; potent antioxidant	Phase I/Italy/HIV	Considered a promising cancer chemopreventive by virtue of its multiple and coordinated mechanisms affecting the process of chemical carcinogenesis (Albini A, etal, International Journal of Cancer, 1995 Mar 29, 61(1):121-9)

ment of malignant recurrent gliomas (brain tumors) and malignant melanoma. The drug exhibited good activity in chemotherapy-naive metastatic melanoma comperable to other most active agents currently in use. In a phase II study, conducted by Cancer Research Campaign in the UK, among 60 chemotherapy-naive (except two) patients with metastatic melanoma treated with temozolamide, 55 of 56 eligible patients were assessable for toxicity and 49 for response. Patients received temozolomide 150 mg/m^2/d over 5 successive days orally (total dose, 750 mg/m^2) in the first course. Courses were repeated every 4 weeks and the dose was escalated to 200 mg/m^2/d x 5 (total dose, 1 g/m^2) after the first course if toxicity was acceptable. CR was documented in three patients (all with lung metastases) and PR in nine patients (21% CR plus PR rate). The overall response rate was 24% and the median response duration was 6 months (range, 2.5 to 22+). Toxicity of the regimen, which was mainly hematopoietic, was low. The median survival duration was 5.5 months (range, 0.5 to 29.5) for all patients and 14.5 months (range, 3 to 28+) for responders, with four patients still alive at the time of the report (Bleehen NM, etal; Journal of Clinical Oncology, 1995 Apr, 13(4):910-3).

ISOLATED LIMB PERFUSION

Renewed interest has developed in isolated limb perfusion (ILP) for the treatment of in-transit and locally advanced recurrent disease because of the availability of melphalan (L-PAM, Alkeran; Burroughs Wellcome) and the recent reports of dramatic clinical responses with the

addition of tumor necrosis factor (TNF)-α and IFN-γ to L-PAM. ILP is performed by surgically isolating the main limb vessels which are then connected to a heart-lung machine. A tight tourniquet is placed around the root of the limb before the administration of high-dose chemotherapy. ILP with melphalan resulted in an average response rate of 50%.

In the TNF-α protocol, pretreatment with 0.2 mg IFN-γ, administered subcutaneously, was used to up-regulate TNF-α receptors. Perfusion was performed for 90 minutes under mild hyperthermia with 4 mg TNF-α for the lower limb or 3 mg for the upper limb. A 91% response rate was achieved with the combined TNF-α and IFN-γ and melphalan regimen. When subgroups were analyzed, the difference remained highly significant. Response rate to ILP using melphalan alone or with TNF-α varied within populations based on disease stage. Patients without lymph node involvement (stage IIIa) experienced a CR rate of 62 % with melphalan alone, compared with 100% for the combination treatment. In those with both in-transit and lymph node metastases (stage IIIab), the rate was 41% compared with 87%. With a median follow-up of 26 months, 23% of patients treated with the TNF-α combination protocol experienced local recurrences, 29% distant metastases, and 17% regional and distant recurrences. Overall median survival was 28 months (Lejeune FJ, ACC95, pp 13-14). Boehringer Ingelheim (Ingelheim am Rhein) is evaluating the use of isolated limb perfusion with TNF-α in sarcoma/malignant melanoma.

MEETING COVERAGE

A REPORT FROM THE 35TH INTERSCIENCE CONFERENCE ON ANTIMICROBIAL AGENTS AND CHEMOTHERAPY (ICAAC), SAN FRANCISCO, CALIFORNIA; SEPTEMBER 17-20, 1995

- Opportunistic infections are rising among cancer patients because of an increase in use of high-dose chemotherapy treatments and bone marrow transplantation procedures; such infections are life threatening, very expensive to treat and represent a very important component in disease management of cancer patients
- In addition to drug-induced neutropenia, cancer patients become vulnerable to infections when chemotherapy is delivered by central venous access catheters
- Fungal infections, against which there are only a few effective treatment options, are emerging as life-threatening complications of aggressive prevention and treatment of bacterial infection
- Also see FO, V1 #5, pp 127-128

BACTERIAL INFECTIONS

Enterococcus faecium (VREF).

One of the growing problems on oncology wards is the increasing amount of colonization and infection by vancomycin-resistant *Enterococcus faecium* (VREF). Pediatric patients colonized with VREF in the stool may continue to shed organisms for weeks to months. These youngsters are at increased risk of developing clinical infections with VREF, a major problem since these organisms exhibit multiple drug resistance (MDR).

Among 305 cancer patients admitted to a pediatric oncology ward in a three month period, 75 (25%) had at least one stool screen and 18 of the 75 (24%) were positive for VREF. Cultures remained positive for a median of 72 days (10-268 days). Three of the 18 colonized patients (17%) developed a clinical infection versus two of 57 children (4%) with negative surveillance stools, a significant difference (Henning J, etal, Abstracts of the 35th ICAAC, Pg. 264:J38).

Improved infection control measures reduced the incidence of vancomycin-resistant enterococci (VRE) on an adult oncology unit. Surveillance of perianal cultures were done on admission and weekly. Patients who were VRE positive were placed on contact isolation, spatially separated from those who were VRE negative, with separate nurses assigned to each group of patients alone. All patients receiving intravenous antibiotics were seen by an infectious diseases physician to cut the use of vancomycin, a broad spectrum antibiotic marketed by Eli Lilly as Vancocin.

Evaluating the patients on a per 1,000 hospital days basis, rates of new patients acquiring VRE dropped to half, from 21.1 per 1,000 days to 10.2 per 1,000 days. Also, patients could be hospitalized longer on the oncology ward before becoming colonized and there were fewer infections. In addition, there was a large reduction in the use of IV vancomycin, going from 703 gm per 1,000 patient hospital days to 430 gm per 1,000 patient hospital days, a 38% reduction in vancomycin use. This is significant since oncologists are used to taking an aggressive approach to treatment and generally used vancomycin empirically.

Patients with severe VREF infections were treated with quinopristin/dalfopristin (Synercid; Rhône-Poulenc Rorer) on a compassionate basis because it is the only effective treatment against VREF but it has not yet been approved for clinical use in the USA. Ordinarily a cure rate of 70% to 75% is seen, a very good rate for resistant enterococcal infections in such a patient population (Montecalvo M, etal, Abstracts of the 35th ICAAC, Pg 264:J39). Synercid, which appears to be effective against pathogens resistant to most other antibiotics, is in phase III trials. The drug, an injectable streptogramin, consists of two semi-synthetic components, dalfopristin (70%) and quinopristin (30%).

Parenthetically, Upjohn (Kalamazoo, MI) is investigating a new class of synthetic antibiotics, the oxazolidi-

nones, that demonstrate similar activity as vancomycin. The company has completed phase I clinical trials with morpholinyl fluorophenyl (U-100592) and is carrying out a similar trial with piperazinyl fluorophenyl (U-100766). The drugs are effective against serious infections involving multi-resistant strains of staphylococci, streptococci and enterococci. Another antibiotic in development addressing VRE infections is Eli Lilly's semi-synthetic glycopeptide LY333328, which has been very promising *in vitro.*

Viridans Streptococcal Bacteremia

Cephalosporin-resistant viridans streptococcal bacteremia is becoming a therapeutic challenge in cancer patients. It appears to be related to the previous administration of β-lactams, a major factor to be considered when selecting empiric antibiotic therapy for this patient population. Between 1991 and 1994, of 370 episodes of bacteremia in cancer patients, 51 episodes were caused by viridans streptococci *(Streptococcus mitis-45, S. salivarius-3, S. sanguis-1, S. anginosus-1, S, mutans-1)*. Of these, 32 (63%) were resistant to ceftazadime (Ceptaz; Glaxo or Tazicef; SmithKline Beecham) and 16 (31%) were resistant to ceftriaxone (Rocephin; Roche) and cefotaxime (Claforan; Hoechst Marion Roussel). The only factor associated with resistant cases was the administration of β-lactam antibiotics during the previous two weeks (4/35 (11%) in ceftriaxone-susceptible episodes and 10/16 (62%) in ceftriaxone-resistant episodes (Carratola J, etal, Abstracts of the 35th ICAAC, Pg 261:J26).

New Therapeutic Modalities for Opportunistic Bacterial Infections

Meropenem. Empiric monotherapy with meropenem (Merrem, Zeneca Pharmaceuticals), a novel carbopenem proving to be useful in clinical situations, is an effective and realistic alternative to standard combination antibiotic therapy in febrile adult and pediatric cancer patients, including those "high risk" individuals with profound and persistent neutropenia. In a large multicenter study conducted by the International Antimicrobial Therapy Cooperative Group of the European Organization for Research and Treatment of Cancer (EORTC) and the GIMEMA (Gruppo Italiano Malattic Ematologiche Maline dell Adulto) Infection Programme, meropenem monotherapy was compared with an established combination of ceftazidime plus amikacin in 958 febrile, persistently granulocytopenic adult and pediatric cancer patients; 483 of whom received meropenem and 475 of whom received the combination. Meropenem was given IV 1.0 gm every eight hours to adults and 20 mg/kg every eight hours to children weighing under 50 kg. Those adults on the combination received ceftazidime IV 2 gm every eight hours, with children getting 35 mg/kg every eight hours; both age groups were treated with amikacin 20 mg/kg as a single dose.

The overall clinical response rates achieved were 56% (270/483) with meropenem monotherapy and 52% (245/475) with ceftazidime plus amikacin. Furthermore, meropenem and combination therapy response rates in 141 children aged 12 or younger were 64% and 62%, respectively. Of particular interest, meropenem was more active than the combination against methicillin-sensitive coagulase-negative staphylococci (which are increasingly important pathogens) with a response rate of 90% (9/10) compared to 47% (7/15) for the combination. As expected, neither regimen was effective against methicillin-resistant strains. Meropenem was well tolerated in both adults and children, with no clinically significant gastrointestinal and no central nervous system toxicity. The overall incidence of adverse events, considered dose-related, was 3.7% for those on meropenem and 6.1% of persons treated with combination therapy (Glauser MP, etal, Carbapenems: New Treatment Options for Infections in Children. Symposium presented at 35th ICAAC by Case Western Reserve University).

Merrem was launched in Italy in October, 1994 in IV and IM formulations and has been approved elsewhere in Europe where it will be marketed as Meronem. Merck's carbapenem Primaxin (imipenem plus cilastatin sodium) was also approved in the UK in March 1995 for the treatment of febrile neutropenia, extending its antibacterial indications. Results of 5-year clinical studies demonstrated the drug may be used as first-line monotherapy before results are obtained from bacterial cultures.

Combination of ciprofloxacin and piperacillin. Combination of ciprofloxacin (Cipro; Bayer/Miles) and piperacillin (Piperacil; Wyeth-Ayerst) has proven to be at lest as effective for febrile neutropenia as the more commonly used piperacillin and gentamicin (Garamycin; Schering), with the added advantage of hastening time to fever resolution, especially in patients with Gram-negative bacteremia. To test the comparative efficacy of gentamicin and ciprofloxacin in empiric antibiotic regimens for patients who have undergone myeloablative therapy and developed fever and infection, 99 persons were randomized to either empiric piperacillin and gentamicin (49 patients) or piperacillin and ciprofloxacin (50 patients). Following antibiotic treatment, 35 patients had negative cultures on piperacillin and gentamicin compared to 41 persons receiving piperacillin and ciprofloxacin, basically comparable. The percentage of patients afebrile within three days, however, was significantly greater in the piperacillin/ciprofloxacin-treated individuals (11%) compared to those on piperacillin and gentamicin (3%). Consequently, vancomycin and amphotericin were required less frequently when piperacillin and ciprofloxacin were initially used (Griggs JJ, etal, Abstracts of the 35th ICAAC, Pg 328:LM8).

VIRAL INFECTIONS

Cytomegalovirus (CMV)

Antiviral treatment of patients with high-grade cytomegalovirus (CMV) antigenemia following autologous

marrow and peripheral blood stem cell transplantation may prove to be useful in preventing CMV pneumonia which occurs in 5% to 9% of CMV seropositive autograft recipients during the first 200 days post-transplant. This is a matter of some significance considering the fatality rate is 63% to 82% in these individuals.

Since only a quarter of patients excrete CMV in their blood, urine, or throat prior to the onset of CMV pneumonia, it was decided to compare the CMV antigenemia assay (AG) with viremia. Sixty seropositive patients were monitored weekly by CMV-AG and for viremia by shell vial and conventional cultures. Antiviral treatment was started only for viremia. Overall, CMV viremia occurred in four patients (7%) an average of 31 days post-transplant, while CMV-AG was detected in 22 persons (37%) an average of 39 days after transplant. Of those, 18 had sporadic episodes of CMV-AG at a level of up to 5+ cells/slide, that resolved without treatment. In contrast, patients who had or progressed to a level of CMV-AG of greater than 5+ cells/slide were at greater risk for CMV pneumonia. Two of four of these patients developed CMV pneumonia, with neither having CMV viremia, suggesting that CMV-AG is more sensitive than the shell vial method and allowing detection eight days before CMV pneumonia onset (Boeckh M, etal, Abstracts of the 35th ICAAC, Pg 202: H128).

New Therapeutic Modalities for Opportunistic Viral Infections

Low-dose intravenous acyclovir (Zovirax; Glaxo Wellcome/Burroughs Wellcome) prophylaxis is a cost-effective way of preventing herpes simplex virus (HSV) infection or reactivation in patients with various types of leukemias such as AML (76%), ALL (16%) and CML (8%) undergoing intensive chemotherapy. Doses as low as 62.5 mg/m^2 every four hours showed efficacy while representing a 50% decrease in drug cost over standard dosing.

To determine a minimal effective dose and schedule of acyclovir in such patients, 517 individuals received 1,000 courses of intravenous prophylactic acyclovir with three consecutive dose and schedule regimens (250 mg/m^2 every eight hours for 309 courses, 125 mg/m^2 every six hours for 225 courses and 62.5 mg/m^2 for 466 courses). The median number of courses was two and the median duration of prophylaxis was 36.9 days (the median duration of bone marrow aplasia). Patients were considered evaluable if they received a minimum of seven days of acyclovir prophylaxis, as well as having all negative surveillance cultures within the first 72 hours of beginning prophylaxis. Of 1,000 courses of prophylactic acyclovir, 998 had no evidence of HSV infection or reactivation, the two failures occurring in the 250 mg/m^2 every eight hours arm (Angelopulos C, etal, Abstracts of the 35th ICAAC, Pg 205:H-143).

Varicella zoster immune globulin prophylaxis. In a study designed to evaluate the impact of varicella zoster immune globulin prophylaxis (VZIG-P) on the hospital course of chickenpox in immunocompromised children, VZIG-P reduced the severity of the disease but did not alter hospital course. Since early initiation of acyclovir therapy resulted in a relatively mild disease with few complications in these immunocompromised children, it may prove to be more cost-effective to give VZV vaccine to household members to prevent chickenpox and provide post-exposure oral acyclovir therapy. These conclusions were reached from a review of 156 immunocompromised children treated for chickenpox with IV acyclovir within a mean 1.7 days after rash onset, with VZIG-P given to 66 (42%), not given to 80 (51%), and unknown for 10 (7%). Half of each treatment group was immunocompromised due to cancer chemotherapy. For the children who did not receive VZG-P, there were more chickenpox-related complications (19/80) and a trend toward multiple and more severe complications compared to those who received VZIG-P (8/66) who usually had one generally mild complication (Law BJ, etal, Abstracts of the 35th ICAAC, Pg 204:H141).

FUNGAL INFECTIONS

According to Francoise Meunier, MD/PhD, director of the Central Office of EORTC (Brussels, Belgium), evidence of invasive fungal infection (fungemia) is present at autopsy in 5% of cancer patients. Approximately 10% to 15% of aggressively-treated lymphoma patients and 30% of cancer patients receiving bone marrow transplants develop invasive fungal infections.

Incidence of a variety of opportunistic fungal infections, including *Candida albicans* and other *Candida* species, *Aspergillus fumigatus, Pneumocystis carinii* and *Cryptococcus neoformans,* among others, has been rising because of increasing populations of immunocompromised hosts. In the USA, the number of hospitalized patients with *Candida* species infections increased 500% during the 1980-1990 decade and the trend continues in the 1990s. Approximately 237,000 hospitalized patients were treated for *Candida*-related infections in the USA in 1993 (see Exhibit 4). *C. albicans* accounts for 8% of all hospital-acquired bloodstream infections but about 35% of such infections are caused by *Candida* species other than *C. albicans.* The most commonly identified new *Candida* species in bloodstream isolates is *Torulopsis glabrata* which is associated with a high treatment complication rate. Others include *C. krusei,* associated with a high mortality rate and *C. lusitaniae,* which is difficult to treat. Changes in the pathogenic spectrum of candidemia may result from increased incidence of immunocompromised hosts, but may also be attributed to increased use of antifungal prophylaxis. Use of systemic antifungals is the single most important risk factor for the development of an antifungal-resistant *Candida* species infection.

More effective drug regimens have significantly reduced mortality rates associated with bloodstream infections around the world from the 80% level that was the norm twenty years ago to 38% to 42% today. Unfortunately,

however, this achievement is dampened by the fact that the incidence of hematogenous candidiasis is on the rise. Critically-ill patients with severe *Candida* infections are usually treated with fluconazole or amphotericin B. Although amphotericin B (Fungizone; Apothecon) is associated with serious toxicity it remains the treatment of choice in severe systemic infections.

In order to reduce its toxicity, amphotericin B has been encapsulated in liposomal formulations allowing delivery of doses 5-8 times higher than free drug, that were tolerated by patients who could not tolerate standard amphotericin B therapy. Three lipid formulations of amphotericin B are currently on the market outside the USA:

- AmBisome (NeXstar; Boulder, CO), a truly liposomal amphotericin B, is a small unilamellar vesical in phase III clinical trials for the treatment of fever of unknown origin, being conducted by Fujisawa USA (Deerfield, IL); it is sold in 16 countries outside the USA, generating revenues of $43 million in 1994
- Amphocil [Amphotec/Amphocil; Sequus Pharmaceuticals (Menlo Park, CA)], a colloidal dispersion of amphotericin B and sodium cholesteryl sulfate, has been launched in the UK and approved in several other European countries for the treatment of systemic infections as a second-line therapy; the drug is licensed to Zeneca in Europe
- Amphotericin B Lipid Complex [ABLC/Abelcet; The Liposome Company (Princeton, NJ)], a combination of two phospholipids, dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidyl-glycerol in a 7:3 molar ratio complexed with amphotericin B in a 50% drug to lipid ratio, is not a true liposome but a so-called "ribbon" lipid structure where a larger volume of amphotericin B than that found in traditional liposomes is interspersed in a lipid bilayer; this product was launched in the UK in April 1995 and the company has submitted an NDA in May 1995 for the treatment of aspergillosis as a second-line therapy

Various azoles are being used in the treatment of fungal infections. Fluconazole (Diflucan; Roerig), a well-tolerated triazole, was effective in the treatment of neutropenic patients (see FO, V1 #5, pp 128-129) with hematogenous infection. Itraconazole is approved for the treatment of aspergillosis that is refractory to amphotericin B.

In a multicenter, 427-patient prospective observational study of candidemia (12% of patients were already treated by fluconazole or amphotericin B when candidemia was detected), *C. krusei* and *C. parapsilosis* were the two most common *Candida* species recovered from patients receiving fluconazole, and *T. glabrata* from those receiving amphotericin B. *Candida* species isolated during episodes of breakthrough fungemia were significantly more likely to be resistant *in vitro* to the antifungal agent being administered. The overall mortality rate associated with candidemia ranged from 35% to 70%, with the highest mortality encountered in patients infected with *C. tropicalis* and *C. krusei* (Ngyuen MH, Focus on Fungal Infections 5, San Francisco, March 9-10, 199%, p 8).

Infection with *Aspergillus* has become one of the leading causes of death from infection during the first 100 days after bone marrow transplantation. Among 2,500 patients transplanted at Fred Hutchinson Cancer Research Center between 1987 and 1993, *Aspergillus* infection was diagnosed in over 214 patients (8.6%). Incidence increased significantly over the five-year study period. The mortality rate was 90%. Eighty percent of infections occurred in the lung; 140 cases (74%) of infection were caused by *Aspergillus fumigatus,* 18 (10%) by *A. flavus,* 12 (7%) by *A. terreus,* and 13 (8%) by *A. niger* (Bowden RA, Focus on Fungal Infections 5, San Francisco, March 9-10, 199%, p 11).

New fungal species are also emerging among AIDS patients, patients receiving transplants and neutropenic patients. In the latter group, new infections such as molds belonging to *Fusarium* and *Trichosporon* species, are becoming increasingly prevalent. Such infections are difficult to diagnose, especially in situations when patients are hosts to several fungal species at any given time. Also various anti-infective regimens administered to profoundly immunocompromised hosts, give rise to drug resistant pathogens. For instance, widespread use of azoles (fluconazole and itraconazole) has increased the frequency of azole-resistant yeasts. Various emerging pathogens have also been identified as resistant to amphotericin B therapy.

Pneumocystis carinii Pneumonia

Pneumocystis carinii pneumonia (PCP) not related to steroid use is increasing significantly in cancer patients, suggesting that it should be considered in all individuals with cancer who receive chemotherapy. A retrospective review of morphologically-proven cases of PCP from 1990 to 1994 was compared to a previous study of 142 cases from 1978 to 1989. Of the 51 patients identified with PCP from 1990 to 1994, steroid use was a risk factor in 37 (71%) versus 116 (87%) of 134 evaluable persons in the earlier study. Of 48 evaluable patients diagnosed ante mortem, 36 (75%) survived versus 58 of 114 (51%) previously reported. Seven individuals failed PCP prophylaxis (three on trimethoprim-sulfamethoxazole started at time of steroid taper, one on aerosol, and three on intravenous pentamidine). Improved diagnosis and treatment modalities have resulted in a significant increase in survival rates, with rates approaching those seen in patients with HIV (Cicogna CE, etal, Abstracts of the 35th ICAAC, Pg 262:J29).

The orally-administered trimethoprim-sulfamethoxasole (Bactrim; Hoffmann-La Roche and Septra; Burroughs Wellcome) antibacterial used in the treatment of PCP in immunocompromised patients was also approved for PCP prophylaxis for the same indication in January 1994. Another agent, trimetrexate glucuronate (NeuTrexin; U.S. Bioscience) was approved as second-line therapy for PCP in immunocompromised patients in December, 1993. RiboGene (Hayward, CA) has initiated clinical trials of RG-201, the company's lead antifungal

Exhibit 4
Incidence of *Candida* Infections in Hospitalized Patients in the USA

Year	First-listed Diagnoses (#)	All-Listed Diagnoses (#)	Change (%)
1989	15,000	147,000	
1990	15,000	150,000	2.0
1991	12,000	183,000	22.0
1992	9,000	204,000	11.5
1993	10,000	237,000	16.2

Source: The National Hospital Discharge Survey of the National Center for Health Statistics (NCHS)

for the treatment of PCP in HIV-infected patients. According to the National Center for Health Statistics, approximately 25,000 individuals were hospitalized for PCP in 1993.

New Therapeutic Modalities for Opportunistic Fungal Infections

Numerous research programs are focusing in the development of more effective and better-tolerated antifungals.

Itraconazole in invasive pulmonary aspergillosis. Prognosis of neutropenic patients infected opportunistically with invasive pulmonary aspergillosis (IPA) can be significantly improved through the use of early chest CT-scans and treatment with itraconazole (Sporonox; Janssen). To reach these conclusions, the features of 50 cases of invasive pulmonary, aspergillosis, occurring in 467 patients with hematologic malignancies from January 1988 to March 1955, were analyzed. The aspergillus antibody test was positive before IPA diagnosis in 23 persons (49%) and aspergillus antigenemia was positive in 17 of 39 tested individuals (44%). A bronchoalveolar lavage was positive in 19 of 34 patients (55.9%). Before October 1991, chest CT-scans were performed in nine of 22 persons and contributed to earlier diagnosis of IPA. After October 1991, early CT-scans were systematically performed in febrile neutropenic patients with pulmonary X-ray infiltrates and it was possible to recognize the CT halo sign in 23 of 25 patients (92%). Median time to IPA diagnosis was reduced from six days to one day.

Forty-two persons were treated with itraconazole 300 to 600 mg daily, with 33 patients (79%) cured or improved with itraconazole alone (15 patients) or, in combination with amphotericin B (18 patients). Twenty three persons were cured (55%), with a median survival of 500 days, and 10 individuals showed improvement (24%), with a median survival of 114 days. Nine patients failed to respond to itraconazole (one pt) alone or in combination with amphotericin B (eight patients) and all died of IPA within the first five weeks of treatment (Caillot D, etal, Abstracts of the 35th ICAAC, Pg 262:J28).

Amphotericin B lipid complex (ABLC/Abelcet; The Liposome Company) has proven to be as effective, but less nephrotoxic than amphotericin B in the treatment of candidiasis. In a 27-center study, 231 patients with hematogenous and invasive candidiasis, 50% of whom had cancer, were randomized to ABLC 5 mg/kg daily or amphotericin B 1.0 mg/kg daily, for a median duration of 14 days. A two-to-one randomization was used, with 153 persons receiving ABLC and 78 patients receiving amphotericin B. The chief causative agent was *Candida albicans* (51%), with the remaining cases being caused by a wide variety of *Candida species.*

Overall response rates were 65% for the 124 evaluable patients on ABLC and 61% for the 70 evaluable patients receiving amphotericin B. At three months follow-up, there were no significant differences in relapse or survival, with 60% of ABLC-treated persons remaining alive versus 51% individuals on amphotericin B. The major differences in the two treatment approaches were in the safety profiles. While the frequency of most adverse events were similar, nephrotoxicity was significantly more common in the amphotericin B group. Baseline serum creatinine doubled in 28% of the patients on ABLC versus 47% of those on Amphotericin B. In addition, the median days to doubling for serum creatinine was 82 days in the ABLC group and 19 days for the patients on amphotericin B (Anaissie EJ, etal, Abstracts of the 35th ICAAC, Pg 330:21).

Liposomal nystatin (Nystatin; Aronex Pharmaceuticals) may represent an alternative to amphotericin B for the treatment of refractory febrile neutropenia in the cancer patient, being as effective and having an excellent renal-sparing toxicity profile at doses up to 6 mg/kg/day. In a phase I clinical trial, 32 adult neutropenic cancer patients with fever refractory to appropriate antibacterial treatment and four days of antifungal therapy were randomly assigned to escalating doses of liposomal nystatin 2 mg/kg/day (9 patients), 4 mg/kg/day (7 patients), 6 mg/kg/day (7 patients), or 8 mg/kg/day (9 patients) for a median duration of eight days. Three patients had documented fungal infections with *Trichophyton glabrate* fungemia, *Aspergillus* pneumonia, and cutaneous alternariosis.

Ten of the 32 patients responded to treatment, the criteria for clinical success being a complete resolution of all signs and symptoms of infection, including a return to

normal for at least 48 hours prior to the end of therapy. Of the 10 patients treated successfully with liposomal nystatin, five had failed fluconazole, one had failed amphotericin B, one had failed amphotericin B plus fluconazole, and three had failed triple combination therapy with amphotericin B, fluconazole, and itraconazole. Drug-related adverse effects such as chills, skin rash, and hypersensitivity reaction did not appear to be dose related but nephrotoxicity at 6 mg/kg/day or over was the dose-limiting toxicity (Boutati EI, etal, Abstracts of the 35th ICAAC, Pg 330:LM22).

BLOODSTREAM INFECTIONS

Bloodstream infections remain a leading cause of death in cancer patients. Factors predicting early death, as identified by multivariate analysis, include age, shock, neutropenia, nosocomial infection, advanced state of cancer, *Pseudomonas aeruginosa* infection, and delay of appropriate treatment.

Nine cancer centers in France and Belgium participated in a five-year survey (1989-1994) of 4,421 episodes of bacteremia in 3,340 patients. Using the Centers for Disease Control (CDC) classification, 61.3% were CDC1 (pathogen isolated), 15.8% were CDC2 (two cultures positive with a contaminant, signs present), 6.9% were CDC3 (one contaminant plus positive signs, positive catheter, positive empiric treatment), and 16.6% were CDC4 (non-significant). The predominant pathogens were coagulase-negative staphylococci (40%), with 19% other Gram-positive organisms, 34% Gram-negative organisms, 2.3% anaerobes, and 3.4% yeasts. Over the five year period, *Enterobacter sp.* increased from 1.0% to 5.0%, while anaerobes decreased from 2.6% to 1.6%. Bacteremias caused by *Candida albicans* dropped from 71% of *Candida* infections to 38%, with *Candida* species remaining stable. According to the attending physician, infection was the cause of death in 21.5% of patients for whom death occurred within a median four days. This compared to death occurring for other causes within a median ten days (Viot M, etal, Abstracts of the 35th ICAAC, Pg 280:J128).

TECHNOLOGY UPDATE

NUCLEAR MEDICINE

- Nuclear medicine's role in diagnosis and treatment of critical diseases has been greatly expanded because of significant technological advances resulting in the development of new radiopharmaceuticals, better imaging systems and expanded applications for existing agents.
- This field is becoming increasingly more specialized, currently focused in at least six major areas, such as oncology, cardiology, pulmonary medicine, neurology, nephrology and orthopedics.
- Information presented here is from the 42nd Anuual Meeting of the Society of Nuclear Medicine which took place in Minneapolis, MN, in June 12-14, 1995.

UNIQUE APPLICATIONS OF NUCLEAR MEDICINE ARE EXPANDING IN ONCOLOGY

Oncology has always been one of nuclear medicine's key application areas. Used both as a diagnostic imaging and therapeutic modality, nuclear medicine provides unique diagnostic information and allows therapeutic interventions not possible with any other competitive technique. Some of the major oncology application areas where nuclear medicine has excelled include:

- Tumor localization
- Tumor staging
- Identification of metastatic sites pre- and intra-operatively
- Assessment of response to therapy
- *In vivo* radioimmunotherapy
- Relief of bone pain caused by cancer

PET/SPECT AND HIGH ENERGY IMAGING

Nuclear medicine has progressed in both SPECT (single photon emission tomography) and PET (positron emission tomography) imaging. With the introduction of more advanced SPECT cameras, sophisticated imaging techniques have become more widely available to nuclear medicine physicians.

Nuclear imaging can be categorized as high energy or low energy, based primarily on the type of radiopharmaceutical used. High energy imaging employs positron emitting agents, such as fluorine-18 deoxyglucose (FDG), a sugar analog that helps visualize patterns of metabolic function, whereas low energy imaging is performed essentially with technetium-based compounds. Parenthetically, FDA approval was granted to Downstate Clinical Positron Emmission Tomography Center at Methodist Medical Center (Peoria, IL) in August 1994 to market FDG.

Currently industry is fighting FDA's 1993 decision to regulate PET radiopharmaceuticals as new drugs, requiring INDs, NDAs and ANDAs in order to clinically evaluate and market these compounds which were exempted from such regulations since 1984. In a Federal Register announcement on February 27, 1995, the FDA solicited comments from the industry and is currently evaluating responses. Industry argues that the unique nature of PET radiopharmaceuticals, namely their short half-life, usually under two hours, and the fact that they are produced and used on-site, distinguishes them from other drugs.

FDG Imaging Based on Cellular Metabolism

Because FDG can be used to indicate cellular metabolism, it is an important tool in identifying neoplasms and assessing tumor growth. Increased availability

of FDG has allowed more widespread clinical use in a broader number of applications. One of the greatest benefits of FDG in oncology has been the ability to distinguish malignant from benign tissue, thereby improving the sensitivity and specificity of diagnosis and reducing the need for unnecessary biopsies.

Evaluation of solitary pulmonary nodules in lung cancer has been one of the unique applications of high energy imaging which has been successful in both identifying the nodules and determining whether they are benign or malignant. Use of this noninvasive nuclear imaging technique could positively impact the diagnosis of most solitary pulmonary nodules. In one multi-center trial, a group of 89 patients, whose chest x-rays showed lung nodules, underwent PET-FDG scanning. Results showed that 85% to 90% of these patients could be evaluated effectively, differentiating malignant from benign nodules, with the malignant tumors exhibiting a higher radiotracer uptake than the benign tumors. According to Dr. R. Edward Coleman, director of radiology and nuclear medicine at Duke University Medical Center (Durham, NC), a negative PET-FDG study could justify a decision not to surgically remove the nodule in the lung. With the increased accuracy provided by PET scanning, about 10,000 patients per year could avoid unnecessary lung surgery. Dr. Coleman believes that results of this clinical trial should convince more third-party payers and managed care organizations to cover the costs of PET scanning.

Whole-body nuclear imaging represents another unique application of FDG-PET imaging. In a recent study at UCLA involving non-small cell carcer (nsclc), whole-body PET scanning with FDG provided additional information that changed the diagnosis and treatment in 35% of patients. Disease staging was originally based on conventional imaging methods, such as x-ray CT, MRI and radionuclide bone scanning. In general, physiologic imaging with PET detected malignancies which were missed by anatomic imaging methods, such as x-ray, CT and MRI. Researchers at UCLA noted that whole-body PET imaging can detect malignancies throughout the body in both soft tissues and bone. In current practice, patients with cancer typically undergo three CT scans (chest, abdomen and pelvis), an MRI brain scan and a radionuclide bone scan to get the same whole-body information. Besides the extra time and cost of so many scans, parts of the body can be missed because there are often anatomic gaps between the chest, abdominal and pelvic CT scans.

Determination of lung cancer that has spread to the mediastinum is important because, generally it is considered inoperable by many physicians. CT has a tendency to show lesions in the mediastinum that are false-positive and physicians must often perform exploratory surgery to determine if the cancer has really spread extensively. However, if the patient undergoes a PET scan that shows malignancy in the mediastinum, an expensive and traumatic thoracotomy procedure can be avoided. Furthermore, if the PET scan shows no radiotracer uptake in the mediastinum, there is more hope that the lung cancer can be surgically treated, according to Dr. J. Madar of UCLA. PET scanning with FDG can aid in visualizing metastatic cancer in the lymph nodes near the lung, permitting the elimination of many mediastinoscopies from the diagnostic workup. In addition to reducing costs, replacing an invasive mediastinoscopy with a noninvasive PET scan is safer and less painful for the patient.

Differentiation between malignant and benign breast tumors was also accomplished by Dr. Norbert Avril and colleagues at the Nuclear Medicine Department at the Technische Universitat (Munich, Germany) utilizing positron emission tomography (PET) with FDG. The group performed PET scanning of 71 patients scheduled for breast surgery. According to the PET results, 37 of the 95 breast lesions were diagnosed as "definitely" malignant, 16 as "probably" malignant and 42 as "unlikely" to be malignant. Biopsy results showed that 49 of the tumors were malignant and 46 were benign. This group concluded that PET scanning of the breast with FDG has a high specificity, indicating that a positive result from the scan is quite reliable for diagnosing malignancy in breast tumors. Furthermore, the sensitivity of PET for detecting breast tumors was 70% to 80%, which is comparable to mammography. Other useful benefits of PET imaging of breast tumors are that it can provide more accurate preoperative staging of the disease. PET can indicate metastatic spread to the lymph nodes in the axilla (armpit) which cannot be detected by mammography. In cases, where PET indicates that the lesion is benign, the clinician may want to follow the disease course for a few months before deciding on surgical resection. Therefore, nuclear medicine results could influence the type of surgery performed and enable physicians to decide which patients are appropriate for breast conservation therapy.

SCINTI-MAMMOGRAPHY AND EVALUATION OF TUMORS IN THE DENSE BREAST

Clinical researchers are cautiously optimistic that advances in nuclear medicine breast imaging may help identify which malignant breast tumors will respond to chemotherapy. There is growing confidence in the ability of radiotracer studies to differentiate benign from malignant tumors, allowing physicians to better decide on the most effective surgical approach for individual patients. These studies may also be useful in determining which patients could benefit from breast conservation therapy.

In the future, nuclear medicine may offer new imaging opportunities that supplement mammography, increasing the sensitivity and specificity of diagnosis. A large multicenter trial is now in progress to evaluate technetium sestamibi (Cardiolite; DuPont Merck) for its ability to image breast tumors. Research investigators ar hopeful that sestamibi will be able to diffrentiate benign from malignant tumors.

Patients with dense breasts pose a dilemma for mammographers because the dense tissue makes it more difficult to see lesions that could be cancerous. Dr. Iraj Kalkhali, director of mammography at Harbor-UCLA Medical Center, has worked with nuclear physicians to develop an effective scinti-mammography procedure using technetium-99 sestamibi. His group carried out a direct comparison of scinti-mammography and mammography in 48 women with dense breasts and suspected breast cancer. Biopsy results showed that these patients had 16 malignant breast tumors and 32 benign lesions. Scinti-mammography correctly identified 15 of the malignant lesions and gave an accurate negative result for 29 of the benign lesions. Mammography, which usually does not distinguish between malignant and benign lesions, detected a total of 30 lesions (of which 13 were malignant and 17 were benign) and mammography failed to detect 18 lesions (of which 3 were malignant and 15 were benign). Based on these findings, Dr. Kalkahli and his colleagues have concluded that scinti-mammography is a good complementary imaging technique to mammography and can improve sensitivity and specificity in the detection of breast cancer in patients with dense breasts.

DETECTION OF TUMORS EXHIBITING MULTIDRUG RESISTANCE

At the Instituto Nazionale Tumori (Naples, Italy), nuclear physicians have found that technetium-99 sestamibi could be useful in showing diffrences between malignant tumors expressing high concentrations of P-glycoprotein (P-gp) associated with mutidrug resistance (MDR) and those that do not. Dr. Andrea Ciarmiello, a nuclear physician at the Instituto Nazionale Tumori, and colleagues studied patients with breast cancer to see if a radiotracer technique could indicate which tumors express high levels of P-gp. Investigators found that the radiotracer clearance or washout rate was a good indicator of the level of P-gp. High levels of P-gp were associated with fast washout rate and low concentrations of P-gp (equivalent to levels found in benign breast lesions) had slow washout rate of technetium-99 sestamibi. This nuclear medicine test could be useful in identifying patients with malignant breast tumors who have a high probability of developing MDR to chemotherapy. For a comprehensive review of MDR in cancer see FO, V1, #2/3 pp74-78, #4 pp 112-115 and #5 pp129-136.

RADIOLABELED SOMATOSTATIN FOR SELECTIVELY TARGETING DISEASE

Somatostatin, a peptide hormone, was first isolated, characterized and synthesized in the early 1970s. Synthetic analogs were described throughout the 1970s and 1980s. One of these synthetic analogs, octreotide, first reported in 1982, has become a breakthrough drug in the treatment of the debilitating symptoms arising from tumors of the gastrointestinal tract and endocrine systems, such as carcinoid syndrome. Receptors for somatostatin are located on a variety of cells, including those of the central and peripheral nervous system, the GI and respiratory tracts, kidneys, activated platelets and lymphocytes, and white blood cells. A variety of tumor types, notably breast cancer and lymphomas (in addition to neuroendocrine tumors) also express somatostatin receptors. These may vary in number and affinity for peptides, but in most cases tumors express somatostatin receptors to a greater degree than do normal tissues.

An additional benefit is the possibility of using the presence and amount of the uptake of radiolabeled octreotide to predict the biochemical and functional characteristics of tumors, helping to predict the response to octreotide therapy. There appears to be a direct correlation between tumor uptake of the tracer and a decrease or elimination of the patient's symptoms that originally resulted from the increased secretion of hormones associated with these tumors. Most neuroendocrine tumors are slow-growing, and their most harmful effects are often related to this hormonal overproduction.

Studies have been under way to quantify peptide uptake by various tumors and correlate it with the response to different therapeutic doses of the octreotide used to control the symptoms. It appears that pretreatment with "cold" somatostatin analog may actually enhance the uptake of the radiotracer in some tumors and that it is not necessary to take most patients off the medication to perform the scintigram.

Some investigators have suggested that a unique benefit of somatostatin analog imaging might be its ability to recognize hormonally silent, undifferentiated endocrine pancreatic cancers, which are often misdiagnosed as pancreatic adenocarcinomas by morphological imaging. This concept may also be useful in identifying pituitary tumors that would benefit from octreotide therapy. Combination of the high spatial resolution of imaging techniques, such as x-ray CT and MRI, with functional peptide scintigraphy could reduce the number and extent of unnecessary surgical interventions and allow appropriate chemotherapeutic treatments to be used, thereby reducing overall cost of care.

More recent investigations and research in tumor imaging have included labeling of peptides with positron emitting tracers, such as fluorine-18 fluoroacylated octreotide, and development of technetium-99 (99mTc)-labeled somatostatin analogs in order to improve image quality.

It is apparent that the applications for peptide scintigraphy will continue to increase, with most clinical interest focused on tumor imaging. Peptides are also in development for imaging infection, inflammation, thrombus and atherosclerosis. There is every indication that lesions can be detected more quickly, safely and inexpensively by peptide scintigraphy than by alternative techniques. These agents also offer the potential for monitoring and verifying the reponse to treatment plans. One exciting prospect is the ability to attach therapeutic drugs, both radioactive and nonradioactive, to these receptor-specific agents, fulfilling the ultimate goal of targeted drug delivery.

Indium-labeled Somatostatin

Mallinckrodt Medical (St. Louis, MO) reduced the problems associated with ^{123}I radiolabeling such as the cost and distribution problems with this tracer, coupled with biliary secretion interfering with abdominal imaging, by using an indium-111 (^{111}I)-labeled somatostatin analog, pentetreotide in its OctreoScan. OctreoScan has been accepted worldwide with more than 12,500 patients studied and led to FDA approval in mid-1994 as the first commercial radiolabeled peptide. (Also see FO, V1 #4, p 90.)

A wide variety of tumors are amenable to imaging with OctreoScan. These include several types of primary brain tumors, pituitary tumors, lung cancer, pancreatic islet cell tumors, gastrinomas, neuroblastomas, pheochromocytomas, carcinoids, paragangliomas, medullary thyroid carcinomas, breast carcinomas, lymphomas, melanomas, Merkel cell (trabecular skin) carcinomas and various head and neck tumors.

CIS-US (Bedford, MA), the USA subsidiary of CIS bio international (Gif sur Yvette, France), is marketing a competitive product to OctreoScan, I-131 MIBG (^{131}I-meta-iodobenzylguanidine sulfate) injection, to localize two types of rare adrenal tumors. This agent has obtained orphan drug status for the detection of pheochromocytoma, a noncancerous tumor and neuroblastoma, a childhood cancer. I-131 MIBG is injected one to two days before scanning and clears the body several days later. Developed at the University of Michigan (Ann Arbor, MI), it was licensed to CIS-US which launched it in June 1994. Like OctreoScan, 1-131 MIBG costs $800 per procedure.

Technetium-labeled Peptides

^{111}I is not an ideal tracer. It has limitations because of the cost of cyclotron production, and is restricted in the amount of material that can be injected into patients to remain within acceptable radiation exposure guidelines. Additionally, ^{111}I emits gamma radiation with multiple photopeaks which are not well suited to current gamma and positron cameras. This leads to diminished spatial and contrast resolution. Finally, OctreoScan has a relatively slow whole-body clearance rate, which delays optimal imaging of the patient for up to 48 hours in some tumors.

Diatech's (Londonderry, NH) ^{99m}Tc-labeled P587 product provides improved imaging characteristics as well as rapid whole-body clearance and effective uptake in tumors. Technetium is also more widely available at much lower cost than ^{111}I. Receptor binding characteristics are similar to OctreoScan, and preliminary results suggest a comparable sensitivity in detecting tumors. Imaging with P587 can be initiated within 30 minutes after injection, which minimizes the interference from abdominal activity often seen with OctreoScan. The photon flux of P587's ^{99m}Tc label is also more compatible with high resolution SPECT techniques. Also, projected costs for the ^{99m}Tc-labeled "techtides" are substantially lower than OctreoScan.

Radiotracers that Bind to Other Somatostatin Receptor Subtypes

Radiotracers that bind to other somatostatin receptor subtypes may be useful in detecting less well differentiated tumors. It appears that there are different, more specific receptors on individual types of tumors that may require a wider selection of radiolabeled peptides to achieve higher sensitivity and specificity. There are at least five subtypes of somatostatin receptors (SSTR 1,2,3,4,5). OctreoScan primarily binds to SSTR-2 receptors, which are abundant in well differentiated tumors.

Note: *This article was prepared by Marvin Burns, President of Bio-Tech Systems (Las Vegas, NV); tel: (702) 456-7608. New Medicine and Bio-Tech systems are currently collaborating in the preparation of a comprehensive report (#202, entitled The U.S. Market for Radiopharmaceuticals) which is scheduled for release in January 1996.*

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