



Anita Philyaw. *Nadine* (detail), 2001. Acrylic on canvas, 18" × 24". Courtesy of Stella Jones Gallery, New Orleans, La.

The role of immunotherapy as an approach for treating patients with renal cell carcinoma is reviewed.

Immunotherapy of Metastatic Renal Cell Cancer

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Background: *The management of metastatic renal cancer remains a therapeutic challenge. Conventional cytotoxic chemotherapy is rarely effective, and the most promising approaches appear to lie in the field of immunotherapy.*

Methods: *The authors review the literature regarding current and investigational immunotherapy approaches to the management of metastatic renal cancer.*

Results: *The mechanism of action, methods of delivery, efficacy, and side effect profile of the cytokines IL-2 and interferon alfa are discussed. The role of investigational approaches such as tumor vaccines, antibody-based therapy, lymphocyte infusions, and bone marrow transplantation is addressed. The rationale for nephrectomy as an adjunctive procedure to immunotherapy is also discussed.*

Conclusions: *Ongoing laboratory investigation of the cause of the immune deficit in patients with metastatic renal cell cancer will result in the development of novel therapies to enhance tumor cell recognition as well as host antitumor response. Translation of laboratory findings into the clinic will be facilitated by the presence of an already well-developed infrastructure for the performance of clinical trials for patients with this difficult diagnosis.*

Introduction

Renal cancer accounts for about 3% of all solid tumors, and its incidence is rising.¹⁻³ Regionally advanced or metastatic cases account for approxi-

mately half of new cases. The management of metastatic renal cancer remains a therapeutic challenge. At the point of diagnosis with metastatic disease, the low response rates (15% to 20%) and even lower cure rates (6% to 8%) of current therapies are discouraging.⁴⁻⁷ Factors such as age, comorbidities, heterogeneous histologies, and especially the potential for a slow natural history can complicate the decision of whether to treat the cancer or to pursue a supportive, symptom-directed approach. The histologic subtype of the cancer has an impact on the chance of response. For instance, the only histologic subtype that appears to respond to immune-directed approaches with any

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consistency is the clear cell subtype. Variants such as granular cell, papillary, or sarcomatoid appear more consistently refractory to the immune approach. Fortunately, clear cell is the most common subtype, accounting for approximately 70% of cases. Earlier successes in applying the immune approach to the problem, as well as the limited contributions from conventional cytotoxic drugs, have helped to make metastatic renal cell cancer a focus for contemporary development of novel immunotherapy.

Cancer as a Problem of Immunity

The immune perspective of cancer differs somewhat from the perspective centered on the cancer cell itself. From the cancer cell viewpoint, the malignant phenotype is a consequence of genetic instability, resistance to apoptosis, unregulated growth, and the capacity to metastasize. In contrast, from an immunological viewpoint, the cancer cell is viewed as a potential target of antibodies, natural killer (NK) cells, or cytotoxic T lymphocytes (CTLs) but for which there is anergy or even specific pathologic tolerance. The presence of tumor in a host logically demonstrates that the immune system and its ability to acquire new, useful immunity either is generally damaged or has become specifically tolerant of the tumor.

Cancer cells have on their surface unique or abnormally expressed antigens for which, in theory, immune specificity could be acquired and to which a clinically useful response could be attained. The rare but dramatic successes of immune therapy highlight some basic questions, the answers to which are the focus of intense research: What are the antigenic features that render some cancers susceptible? Are there genetic features in particular individuals that could predict responsiveness? What cancer genetic features are causative for the resistant phenotype? Studies to answer these questions and to investigate the mechanism of the immune deficit are ongoing. Some features under study include T-lymphocyte dysfunction,⁸⁻¹⁰ dendritic cell dysfunction in advanced cancer,¹¹ identification of unique antigens for which immunity is inducible,^{12,13} and vaccine manipulations to circumvent or defeat tolerance.

Rationale for and Contribution of Nephrectomy to Immunotherapy

The role of nephrectomy in patients with metastatic renal cell carcinoma is controversial. Insofar as the primary tumor bulk has a negative effect on the immune system, its removal can be considered to have

immunotherapeutic potential. The characterization of the mediators of this negative effect is ongoing. Prior to the introduction of immunotherapy, the prognosis of patients with metastatic renal carcinoma was dismal, with reported 1-year survival rates of 26% and 3-year survival rates of 4%.¹⁴ In these circumstances, nephrectomy added little to patient management, and there was general agreement that surgery was indicated only for symptom palliation for patients with a good performance status.¹⁵⁻¹⁷ An exception to this practice was for patients with a solitary metastasis in whom prolonged survival can occur following a combination of nephrectomy and surgical resection of the metastasis.¹⁸⁻²⁰

More recently, the clinical algorithm for metastatic renal cell cancer has switched in favor of early nephrectomy for patients with a good performance status. There are several reasons for this change.

First, two similar trials, SWOG 8949²¹ and EORTC 30947,²² compared nephrectomy plus interferon alfa (IFN- α) to IFN- α alone in patients with metastatic renal cancer. The EORTC trial reported a 10-month median survival advantage in the combination group compared with the IFN- α alone group (17 months vs 7 months), and the SWOG trial noted a 3-month median survival advantage for the combination group (11.1 months vs 8.1 months).

Second, a better understanding of prognostic factor in patients with metastatic renal cancer allows selection of patients who are likely to have a more prolonged survival and therefore a greater likelihood of benefit from surgery.²³ A variety of prognostic factors have been identified, and their influence on survival is undoubtedly greater than many treatment considerations. Several reports on these factors have been published.^{4,18,23,24}

Third, improved surgical techniques mean that nephrectomy can be performed with lower morbidity, ensuring that a high proportion (upwards of 80%) of patients proceed to systemic therapies.²⁵ A recent report suggests that even patients with locally advanced disease (T3 with vena caval thrombi) can safely undergo nephrectomy with 80% receiving subsequent immunotherapy.²⁵ Improvements in technology are likely to further decrease the morbidity associated with nephrectomy and speed the time to commencing systemic therapy. Walther et al²⁶ recently reported a series of patients undergoing successful laparoscopic cytoreductive nephrectomy for moderate-sized renal tumors (a median diameter of 9 cm). Patients undergoing laparoscopic nephrectomy had less postoperative pain, a faster time to discharge, and a shorter time to

interleukin-2 (IL-2) treatment than a similar group of patients who had an open nephrectomy.

Fourth, several studies have demonstrated that the primary tumor rarely responds to IL-2-based therapies.^{21,27} Wagner and colleagues²⁷ reviewed 51 patients treated at the National Institutes of Health with the primary tumor in situ and documented no responses in the primary tumor. Reports of significant tumor reductions that facilitate surgery are even rarer, with some authors suggesting that a nephrectomy following a response is more difficult due to the treatment-associated fibrosis and scarring.²⁷

Finally, there is the biological argument, supported by older data from animal models, that the large bulk of the primary tumor is either immunosuppressive or acts as an immunological sink with a suppression of cell-mediated immunity that is reversed upon removal of the primary tumor.^{28,29} Human studies have shown changes in cell-mediated immunity after nephrectomy,³⁰ with a recent retrospective study reporting lower than expected systemic antitumor responses when the patients were treated with the primary tumor in situ (6% vs 22% to 35%).²⁷

In summary, a proven (albeit small) survival advantage, decreased surgical morbidity, and a theoretical biological benefit have reawakened the interest in surgery as an important part of the multidisciplinary management of metastatic renal cell carcinoma. Integration of this important treatment decision with subsequent plans for immunotherapy and individualized risk assessment is necessary.

Therapy Based on Available Cytokines

The two principal cytokines available as noninvestigational agents for renal cancer therapy are IFN- α and IL-2. Dosage is measured as millions of units (mIU). IFN- α is usually administered via subcutaneous injection, generally on an outpatient basis. IL-2 is administered either by the subcutaneous route or intravenously, usually in the inpatient setting with the higher doses. Prominent cytokine side effects can interfere with delivery of planned drug doses and necessitate hospitalization and ICU admission for supportive care. There are many published route and combination schedules, some of which are discussed here. Response rates vary across different series. The variation may be selection bias or chance variation. The incidence of side effects is dose related, so many schedule innovations are designed with the intention of preserving (but not improving) response rate while decreasing toxicity.

Intravenous IL-2

The Food and Drug Administration (FDA) has approved IL-2 for the treatment of metastatic renal cell cancer at a dose of 600,000 IU/kg (approximately 40 to 50 mIU) per dose, administered in a slow intravenous infusion every 8 hours as tolerated to a maximum of 14 doses per cycle.³¹ A variety of other dosing schedules have been used, including 720,000 IU/kg per dose,^{31,32} 72,000 IU/kg per dose (intermediate dose, inpatient but not ICU setting)³³ and 18 mIU/m² per day given as continuous infusion for 5 days.³⁴ Randomized trial data have suggested bolus and continuous infusions are comparable in efficacy and toxicity.³³ The total dose per course in the continuous infusion schedule is usually less than the 600,000 IU/kg per dose bolus schedule indicated by the FDA. The high-dose intravenous schedules are associated with significantly more prominent side effects and require hospitalization, generally in a ward with capabilities for dopamine infusion and close monitoring of blood pressure.³¹⁻³⁵ Because of the toxicity issues, as well as the necessity for availability of pressor (dopamine) infusion, the regimen is frequently perceived as cumbersome and risky. Fig 1 shows the changes in albumin and creatinine in a patient receiving IL-2, illustrating the significant physiological changes associated with such therapy.³⁶ Restricted use of high-dose intravenous IL-2 to referral or high-volume centers is a practice pattern that may be seen for this FDA-indicated drug schedule.

Subcutaneous IL-2

Subcutaneous lower-dose IL-2 regimens have been an active area of development. In an effort to

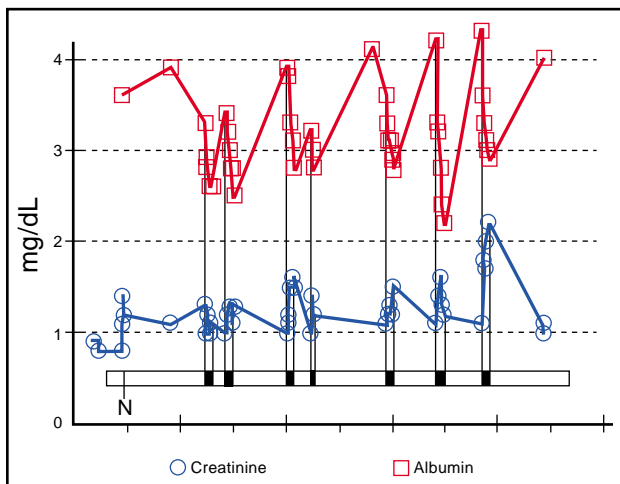


Fig 1. — Illustration of the fluctuation of serum albumin and creatinine in a patient, reflecting capillary leak syndrome. This patient received 7 cycles of high-dose IL-2 and IFN- α (as in Négrier et al³⁶) for lung-metastatic renal cell carcinoma, with complete response in chest. The upper line shows serial albumin measurements, and the lower line shows serial creatinine measurements. Lower bar: N = date of nephrectomy. Shaded areas: IL-2 infusion days (5-day cycles, one is 3 days). Each tick on the horizontal axis is 30 days.

improve tolerability, these regimens often result in more treatment days but not hospitalization, or they may require inpatient treatment but not ICU admission.³³ Combinations with IFN- α and 5-FU have also been emphasized. Several phase II series describe a variety of regimens (some of which are compared by Négrier et al³⁶), each with some responses. Fig 2 illustrates a comparison of schedules used in three combinations of 5-FU, IL-2, and IFN- α that have been reported with divergent response rates.³⁶⁻³⁸ A direct, randomized comparison of high-dose inpatient IL-2 and an outpatient schedule restricted to patients who would otherwise be eligible for inpatient high-dose IL-2 has been presented (favoring the high-dose cohort, discussed later).³⁹ Improved tolerability would allow more patients to consider starting, as well as successfully complete, the treatment courses.

Interferon

IFN- α is approved in Europe for treatment of renal cell cancer. There is a significant base of experience in clinical trials.^{40,41} Side effects also are dose limiting, including use as a single agent given on an outpatient regimen or in more complex combinations. The broad range of toxicities can overlap those of IL-2 or other partner drugs. These associated side effects include a flu-like syndrome (fatigue, fever, chills, anorexia, myalgia, headache, arthralgias, and diaphoresis), dry skin and mucous membranes, mental status changes and depression, and other side effects at lower frequency. Consideration for prophylactic attention to these problems should be considered routine. Acetaminophen and diphenhydramine improve IFN- α and IL-2 tolerability. Prophylactic management of the depression-inducing

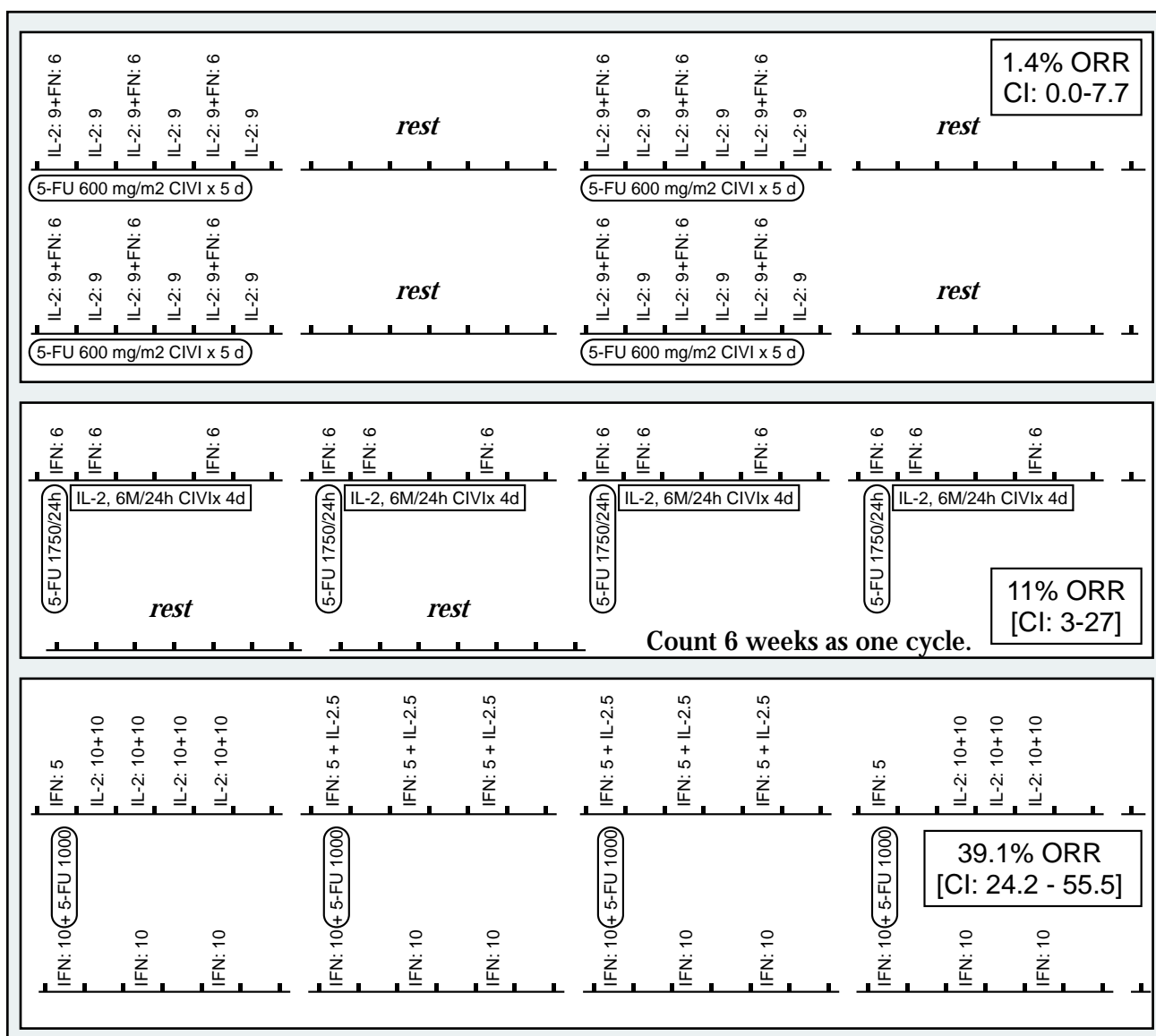


Fig 2. — Illustration of three different combination treatment schedules using 5-FU, IL-2, and IFN and their reported response rates from independent series. Upper: Négrier et al.³⁶ Middle: Elias et al.³⁷ Lower: Atzpodien et al.³⁸

potential using anti-depression drugs has been an active point of discussion, especially in relation to other IFN indications.⁴²

The effect on measurable tumor dimensions is not especially rapid. The median time of onset of partial response (PR) was 67 days among patients achieving PR in the 8 studies reviewed in the Proleukin (IL-2) FDA application³⁶; latency to complete response (CR) was longer. The selected outpatient-based cytokine schedules and the associated response rates have been reported by Négrier et al.³⁶

How Cytokines Work

Although the exact mechanism of action of cytokines is complex and unknown in a usual immunotherapy model, the effect of the drugs on the cancers cells is indirect. The cytokines bind to their specific receptors and initiate intracellular and intercellular signaling cascades. IL-2 is a potent stimulator of T-cell proliferation, and if antitumor T cells are present, they could be stimulated. The consequence of this action is that tumor-specific CTLs, NK cells, and possibly the subset of these that are intratumoral (tumor-infiltrating lymphocytes) are activated, and these leukocytes then kill the cancer cells.

The approved IL-2 indication is based on the effect on T lymphocytes.³¹ However, renal cancer cells themselves bear receptors for these (and other) cytokines and may secrete soluble IL-2 receptor that affects the interaction of the IL-2 with the lymphocyte IL-2 receptor. In the clinical setting, it is not realistic to try to separate the effects on tumor cell IFN receptor,^{43,44} tumor cell IL-2 receptor,^{45,46} soluble IL-2 receptor, or CTL and NK subsets. Suffice to say the “conventional anticancer drug” direct-action mechanism may have a role in some of the responses that are observed. Antiangiogenic effects of IFN (α , β , or γ) have also been observed in different clinical circumstances.^{47,48}

Quantifying Immune Therapy Benefit

The successes of renal cancer immunotherapy in curing or improving survival is restricted to a narrow subset of the metastatic cancer patient population.^{24,49} In the decision to proceed with a trial of immune therapy for an individual, the side effect risk might work against the third goal of therapy in advanced cancer: palliation even in the absence of improved survival. Quality of life metrics have been incorporated into some newer studies. Estimates of CR and PR are generally in the 6% to 8% and 15% to 20% range, respec-

tively, in larger series.⁴⁷ Smaller series with better rates, such as reported by Atzpodien et al,³⁸ appear difficult to reproduce, again probably related to case selection. Long-term survivorship is concentrated in subjects with CRs, and some improvement is also observed for PRs.^{6,7} The survival benefit of immunotherapy-induced stable disease, however, is questionable, if not absent.⁶ Retrospective analyses of immune therapy as a prognostic point consistently indicate the immunotherapy treatment (as well as nephrectomy status, see above) are independent prognosticators for longer survival.^{23,24,49}

Our understanding of patient or cancer features that portend success is incomplete. Some laboratory and clinical features that favor longer survival or higher response frequency have been identified in retrospective series. These include absence of anemia, absence of hypercalcemia, normal lactate dehydrogenase (LDH), a prior nephrectomy, and good performance status.²³ A better understanding of pretreatment prognostic factors and possibly tumor- or patient-specific genetic factors may provide more precision as to how an individual patient compares with the group treated on a single-arm trial.

Phase III Outcomes

Positive data from recent randomized, controlled trials include the comparison of the combination of IFN- α (up to 18 mIU units 3 times a week) plus vinblastine to vinblastine alone, favoring the combination (overall response rate: 16.5% vs 2.5%, $P=.0025$; median survival: 67.6 weeks vs 37.8 weeks, $P=.0049$),⁴¹ and the SWOG 8949 and EORTC 30947 nephrectomy trials discussed above.^{21,22} An early report on the randomized trial between high-dose IL-2 vs outpatient IL-2 plus IFN- α (at a relatively low dose of IL-2 of 5 mIU/m²) was reported at the 2001 meeting of the American Society of Clinical Oncology.³⁶ The higher schedule showed a trend toward improved overall responses, with 25 of 99 patients in the high-dose arm responding vs 12 of 94 patients in the combination arm. Final published data are awaited. Similarly, data from the Medical Research Council trial of IFN- α (10 mIU 3 times a week) vs medroxyprogesterone (300 mg/day) favored the IFN- α arm. Median survival was, respectively, 9 months vs 6 $\frac{3}{4}$ months, the 1-year survival rate was 43% vs 32%, and the 2-year survival rate was 22% vs 13%.^{50,51} The relatively smaller (but randomized) trial reported by Atzpodien et al³⁸ illustrated a promising outpatient combination schedule, CR + PR = 39.1% (95% confidence interval [CI], 24.2% to 55.5%). The comparator arm was also “hormonal” (ie, tamoxifen); among 37 patients receiving tamoxifen, no objective responses

occurred, but 13 patients (35.1%) had stable disease. The retesting of the effect of this schedule against a control arm using a different cytokine combination schedule could be confirmatory.

Negative data from randomized, controlled trials include the trial reported by Négrier et al³⁶ in December 2000 that compared an outpatient IL-2 and IFN- α regimen (which used relatively lower IL-2 doses) with or without the addition of 5-FU for two 5-day courses. Each arm had a low response rate overall. The adjuvant use of IFN following metastasectomy has not been tested, but its use on an adjuvant schedule in high-risk patients who did not have metastatic cancer has been reported and appears not useful.⁵²

A trial of the addition of 13-*cis*-retinoic acid to an outpatient schedule of IFN- α was reported by Motzer et al.⁵³ The combination arm had a 12% vs 6% ($P=.14$) overall response rate, 19% of patients in the combination arm were progression-free at 24 months compared with 10% in the second arm, and longer duration of responses were achieved (33 vs 22 months, $P=.03$). However, median survival was comparable ($P=.26$), and quality of life was poorer for the combination arm, especially during the first 8 weeks of treatment. A trial comparing tamoxifen 40 mg with or without IFN- α and IL-2 was closed to accrual when interim evaluation showed no survival difference, and final evaluation confirmed that result.⁵⁴

A three-arm trial by Négrier et al³⁴ compared subcutaneous IL-2 (18 mIU/m² per day via continuous intravenous infusion for four 5-day courses) vs IFN- α (18 mIU given 3 times per week) vs a combination of IL-2 (same) and subcutaneous IFN- α (6 mIU given 3 times per week). Higher response rates were achieved in the combination arm at week 10 (7.7% vs 7.8% vs 21.3% of the evaluable patients, $P=.01$), and at week 25 (2.9% vs 6.1% vs 13.6%, $P=.001$). Overall survival (log rank) was *not* significantly different ($P=.55$), although the median survival appeared to favor the combination (12 vs 13 vs 17 months). Also, the combination arm had the most toxicity. Interestingly, patients with only one metastatic site who received both cytokines had a 37% probability of a response.

An ongoing ECOG trial (E2898) is a phase III trial of IFN- α 2b alone vs IFN- α 2b plus thalidomide in patients with untreated metastatic or unresectable renal cell cancer. Both agents have proposed angiogenesis and immune modulation effects.⁵⁵⁻⁵⁷ A study objective is to address the antiangiogenesis mechanism. An understanding of the relevant mechanisms by which these drugs act will be needed to formulate a rational extension of these results.

Variants of IL-2 and IFN

The problematic effects of systemic administration have been part of the basis for development of variant cytokines. These variant molecules are based on IFN- α and IL-2 but with additional pharmacologic design features beyond duplication of the natural compound, especially to avoid systemic toxicity.

Leuvectin is a product in which a plasmid DNA expression vector coding for the human IL-2 protein is contained in a cationic lipid that promotes DNA transfection. The rationale for its development is to maintain the efficacy of IL-2 while minimizing systemic side effects by placing IL-2 production locally in the tumor and thereby inducing its antitumor activity locally.^{58,59} This concept relies on the possibility of systemic spread of the immune response to other metastases besides those directly reachable by injection. Early phase trials are open and require a tumor site that is accessible for injection.

Pegylated interferon (PEG-Intron) has approximately a 10-fold greater maximal concentration and a 50-fold greater area under curve (AUC) than IFN- α 2b.⁶⁰ The once-a-week dosing schedule may provide longer sustained active IFN- α levels. The published base of experience with PEG-Intron in renal cell carcinoma is limited to one phase I study.⁶⁰ Responses have been observed and further testing will be useful in determining if there are significant efficacy or toxicity advantages over conventional IFN- α . PEG-modified IL-2 has also been tested in a phase I trial (combined with standard IL-2) for patients with renal cell carcinoma.⁶¹ A liposomal IL-2 has been developed.⁶² The issue of whether only pharmacokinetics and dosing frequency are improved or whether an improved immune modulation occurs for these agents is not settled.

Through targeted mutagenesis, a synthetic form of IL-2 has been developed that has approximately a 3,000-fold selectivity for T cells (putative useful effectors) than for NK cells (putative cause of IL-2 intolerance side effects). This variant IL-2 BAY 50-4798 (Bayer Corp) is in early clinical development (open phase I trial).⁶³

Other Cytokines

Other cytokines, also members of the IFN and IL families, are known to be involved in immune signaling. Several have been manufactured in clinical quantities and tested in renal cell cancer.

IFN- γ is produced by antigen-stimulated T lymphocytes. Its production may be impaired in renal cell can-

cer.⁸ In vitro, IL-2 and IFN- γ act synergistically to generate lymphokine-activated killer cells; however, immune modulation does not necessarily imply clinical utility.⁶⁴ Phase II evaluations for renal cell cancer therapy in IL-2 combinations had 10% to 11% objective response rates,^{65,66} but a study on a 5-FU combination had best response of stable disease with no major responses.⁶⁷ In another trial, placebo treatment was comparable.⁶⁸ The IFN- α plus IFN- γ pair has also been explored in conjunction with autologous tumor vaccine.⁶⁹

IL-6 is a cytokine that may be elevated in advanced renal cell cancer. Pharmacologic administration of IL-6 was explored in a phase I trial also using granulocyte-macrophage colony-stimulating factor (GM-CSF). The treatments were safe, but further development for the renal cell cancer application was not recommended.⁷⁰ IL-12 is a cytokine in active early clinical development for the renal cell cancer application. IL-12 can induce IFN- γ production by T lymphocytes.⁸ Activity and synergy with IL-2 or with IFN in the Renca murine renal cancer model and frequent disease stabilization in phase I trials are encouraging.^{71,72} Open trials address issues such as combination with high-dose IL-2. Hematologic toxicity (eg, agranulocytosis and hemolysis) also needs to be addressed in the further development of IL-12.⁷³

Vaccines to Modulate Immunity

While cytokine therapy activates the immune system, vaccine therapy seeks to direct the immune system specifically to the tumor cell targets. Once the immune system, especially the CTLs, are correctly oriented, a nonspecific activation signal such as a cytokine could be more effective. The first empiric step in clinical development of a vaccine is demonstrating the acquisition or amplification of CTLs with antitumor specificity. The process of antigen presentation to lymphocytes is a key step in development of lymphocytes with specificity for a particular tumor antigen. The next crucial step is to get the CTLs to act on the tumor.

The presence of tumor at a particular point in time is logical evidence that the status quo immune surveillance is inadequate. The inadequacy may reflect a general immune suppression, as described for lymphocytes or dendritic cells of advanced cancer patients,⁸⁻¹¹ or may be a tumor-directed, pathologic, immune-tolerant, anergic state of tumor-specific CTL clones. In the rare instances when resection of the primary leads to "spontaneous" regression of distant metastases, one would suppose that the immune system had the capacity to attack the metastases all along but was prevented by something that was removed when the primary tumor was excised. The goal of vaccine therapy is to defeat

pathologic anergy by stimulating quiescent CTL clones into the active antitumor state.

All cells, including tumor cells, bear major histocompatibility complex (MHC) molecules on their surface that can display peptides for immune surveillance. The peptide/MHC can interact with a naive T lymphocyte. The important feature of the interaction of a T cell and a cell presenting a peptide antigen/MHC molecule is whether the T cell is induced to become activated or anergic. Specialized antigen presenting cells, particularly dendritic cells, are able to present antigens in the context of the correct co-stimulatory molecules so as to produce activation, not anergy. If a naive T cell encounters antigen in a context without the co-stimulatory molecules, anergy is favored, similar to the process of normal, physiologic self-tolerance. If the same antigen were to be presented in the context of appropriate co-stimulatory molecules (eg, GM-CSF, CD-40, and B7.1), activation could be produced.

At present, no licensed vaccines are available for renal cancer. (Melacine, a melanoma vaccine, is licensed in Canada.) This illustrates the fact that the process of demonstrating survival benefit or objective tumor response is more difficult than demonstrating the laboratory objective of inducing CTL with tumor specificity. Several innovative approaches have been reported or are being evaluated in ongoing clinical trials, as described below.

Vaccination with the peptide corresponding to a particular von Hippel-Lindau (VHL) mutation is one. The VHL gene, located on human chromosome 3, appears to be an early genetic alteration of renal cell cancer. Approximately 50% of cases have a mutated gene, and another 25% have some other silencing of the pathway.^{72,74} The principal cancer observed in individuals with an inherited mutation in one allele (VHL syndrome) is clear cell renal cancer, in addition to cerebellar and retinal hemangiomas and pheochromocytoma. Pathologically, defective VHL protein function leads to upregulation of genes whose transcription is promoted by hypoxia-inducible factor (HIF)-1 α , and this appears to be a key feature in the onset of the malignant phenotype. In an open trial at the National Cancer Institute,¹³ individuals whose tumor has a VHL mutation are immunized with the particular mutation (which is an antigen unique to the tumor) with a keyhole limpet hemocyanin (KLH) adjuvant. This single-peptide vaccination approach may be contrasted with whole-protein or whole-cell approaches.

A cell's antigenic peptides are associated with the heat shock protein HSP96. The Oncophage product (Antigenics Inc) uses this autologous tumor-specific set

of peptides and is being tested in two placebo-controlled, randomized phase III trials, one for stage III disease and one for stage IV disease.^{75,76}

In whole-cell approaches, the tumor cell itself is used to provide the broadest set of tumor-related antigens. Transducing the tumor with genes so that the tumor cell may act like an antigen-presenting cell⁷⁷ or may attract and stimulate local antigen-presenting cells⁷⁸ are two approaches. Currently at our center, an open phase II trial is using a B7-1 transduced autologous tumor vaccine in concert with outpatient schedule subcutaneous IL-2. B7-1 is a cell-surface co-stimulatory molecule found on antigen presenting cells (but not naturally on tumor cells) that mediates interaction with lymphocytes to effect stimulation instead of anergy. The phase I report describes 4 of 19 responses that cannot be necessarily attributed to the vaccine vs the IL-2 components in such a small series.⁷⁷ Evidence of induced immunity (ie, conversion of delayed-type hypersensitivity [DTH] testing to positive) was seen in this series and correlated with a clinical antitumor response. This DTH conversion was also observed in a GM-CSF-transduced vaccine study.⁷⁸ Simons et al⁷⁸ tested GM-CSF-transduced autologous tumor as vaccine in several genitourinary cancers, including renal cell carcinoma. The GM-CSF may act to recruit and stimulate endogenous antigen-presenting cells to process antigen from the tumor cells.

Antibody Immunotherapy

The products administered in the cytokine- and vaccine-based paradigms use indirect approaches to stimulate the immune system to attack the tumor. Another approach to utilize the immune system is to directly administer a natural or altered antibody. The conceptually simplest of these antibodies attack a particular cell-surface protein. The G250 antigen (which is the same as the cellular protein MN/CA9) is specifically expressed on most renal cancers.⁷⁹ Early-phase clinical trials with unlabeled or ¹³¹I-labeled radioactive G250 antibody, which is specific for this antigen, showed some stable disease responses.^{80,81}

ABX-EGF is another antibody in early development. The antigen EGFR (epidermal growth factor receptor) is a receptor tyrosine kinase whose ligands include EGF and transforming growth factor α . EGFR is overexpressed on renal cell carcinoma⁸² and many other cancers. Whereas an EGFR tyrosine kinase blocker (such as Iressa) blocks the function of this tyrosine kinase within the cells (small molecule direct targeting approach), EGFR-directed antibody may block the function by preventing ligation, but it also can mark the overexpressing

cells for immune attack. Frequent stable disease/minor responses (58%) and some partial responses (6%) have been described.⁸³

Vascular endothelial growth factor (VEGF) is a protein whose blood levels may be increased in advanced cancer. It is the ligand of the VEGF receptors, which are tyrosine kinase enzymes, and an apparent angiogenic factor. In murine systems, tumor-induced production of VEGF or its infusion by a slow-release pump results in impaired dendritic cell function,⁸⁴ and conversely, depletion with antibody promotes the function of dendritic cells.⁸⁵ The antibody bevacizumab (Avastin) depletes VEGF and is in clinical trials, including for renal cell cancer. The randomized phase II trial at the National Institutes of Health (comparing placebo, low-dose, and high-dose) showed improved time to progression (hazard ratio 2.3, $P=.001$) but only 3 objective responses in 110 patients, all of which occurred in the high-dose group.^{86,87}

HuKS-IL2 is a fusion protein of a tumor-targeting antibody and a cytokine. The humanized antibody specific for epithelial cellular adhesion molecule KSA is fused to the IL-2 protein. Many tumor cell types bear the KS antigen on their surface, and lymphocytes bear high-affinity IL-2 receptors, which allows the drug to concentrate cytokine activity at the vicinity of the cancer cells. A phase I trial has enrolled patients and closed.^{88,89} The antibody L19 (whose antigen is a fibronectin isoform associated with tumor angiogenesis) also has been fused to IL-2, with favorable results in a mouse model.⁹⁰

Other antibody approaches have been used to target radioactivity, cytotoxic drugs, cytotoxic chemotherapy, or toxins. These drugs generally are available only in the framework of clinical trials, and drug supply may be a limiting factor in the pace of their development.

Exogenous Lymphocyte Infusion

Tumor-infiltrating lymphocytes (TILs) are an appealing subset of the lymphocyte pool in that they represent clones that at least target the correct location. Expansion of the lymphocytes obtained from the primary tumor, using *ex vivo* recombinant IL-2, and reinfusion in conjunction with cytokine therapy was of interest when a 33% response rate was observed in a phase II series.⁹¹ Subsequently, a randomized, controlled trial was conducted using nephrectomy, blinded TIL/placebo infusion, and intravenous (5 mIU/m²) IL-2 therapy. However, the trial did not demonstrate utility of the newer technique (response rate 9.9% vs 11.4% favoring the control arm).⁹² The factors underlying the

ineffectiveness of the addition of TILs to IL-2-based therapy are not understood, although in this trial many patients either did not receive therapy after the nephrectomy or did not have successful production of the TILs. Also the IL-2 dose was lower than that used in some IL-2 trials. Variations in the preparation of TILs or the preparation of the host immune system to accommodate the TILs continue to generate some interest. At the minimum, the TILs represent the CTL clones that are thought to be at the core of effective antitumor immunity, even though this expansion and reinfusion technique did not demonstrate an advantage, perhaps a consequence of a tumor property causing the TILs to be neutralized.

Can *ex vivo* activation of lymphocytes render them competent to overcome suppressive forces within the host? Lymphokine-activated killer (LAK) cells is a term used to refer to a T-lymphocyte subset that can be measured from the peripheral blood of patients receiving cytokine treatment or to exogenously activated lymphocytes that are then reinfused, usually in conjunction with cytokine therapy. The process of exogenous activation of the lymphocytes obtained as an apheresis product is principally by short-term culture incorporating exposure to IL-2, although other *ex vivo* cytokine combinations have been evaluated. Initial enthusiasm from an apparent high frequency of response in small single-arm series^{93,94} waned as larger randomized series showed an absence of sustained benefit with the addition of the LAK component to the therapy and even the potential for enhanced toxicity.^{5,95,96}

If the native immune system cannot be stimulated, primed, or augmented to reject renal cancer, could a foreign one be used? The technique of allogeneic mini-transplantation uses an immunologically similar but not identical donor. The MHC genes are located in a tightly linked locus so the lymphocytes of a matched (6/6) sibling will have no major (but likely some minor) incompatibility. Each additional (full) sibling has a 25% chance of being a match, but there are intrinsic limitations on the proportion of patients who will have a suitable donor. Among 84 metastatic renal cell carcinoma patients with siblings, representing 29.6% of 284 such patients seen during that period, Rini et al⁹⁷ identified 37 who did not have a match and 23 who had rapidly progressive cancer, leaving only 24 (8%) who were eligible for enrollment for the procedure.

In this technique, the immune system of the host, having already failed to clear the cancer, is suppressed with conventional cytotoxic drugs to the extent that the donor graft, generally obtained from a peripheral blood stem cell collection product, can become established in the host. The choice of drugs for the condi-

tioning of the host to achieve chimerism appears important. Similarly, the fractionation of the donor graft product (stem cells vs lymphocytes), delayed donor lymphocyte infusions (DLIs), and the timing of the withdrawal of immunosuppressive drugs are factors that affect how the graft establishes itself in the donor. Once the chimeric marrow state is established, the lymphocytes of graft origin (which could theoretically be augmented with DLI) may act against host tissues.

This allogeneic immune attack may be conceptually divided into graft-vs-host and graft-vs-tumor. Acute or chronic graft-vs-host may represent a life-threatening condition. If the graft-vs-host disease is manageable through careful monitoring and titration of immunosuppressive drugs, the graft-vs-tumor effect has curative potential. Two series have been published on this approach. In the first, Childs et al⁹⁸ treated 19 patients, all of whom had renal cell cancer refractory to IL-2-based immunotherapy. CRs occurred in 3 patients and PRs in 7 (one of whom died of infection). More recently, Rini et al⁹⁷ treated 15 patients, with 4 partial responses but also 4 treatment-related mortalities. Confirmatory trials are open at several centers, and variations on the technique will be addressed, particularly choice of conditioning regimen and timing of DLI.

Conclusions

The field of immune therapy of renal cell cancer is growing in many directions. The potential for a positive contribution from tumor bulk removal (nephrectomy) is recognized. However, the mechanism for the benefit of debulking is uncertain. The efficacy and side effect profile of the commonly used cytokines IL-2 and IFN- α are discussed. The low frequencies of useful response that can be attained with these older cytokine and/or chemotherapy combinations have been explored in studies encompassing thousands of patients. Although regimens with better toxicity may be identified using ongoing phase III trials or predictors of response based on clinical factors may be identified, consistent improvements of response frequency have not been attained. Exploration of new agents, more so than new schedules of old agents, could address this issue.

The need to extend the dramatically good results in the occasional patient that initially engendered the interest in immunotherapy to the broad range of patients with metastatic kidney cancer is clear. The infrastructure to perform clinical trials for these patients is well established. Better basic science insight into the immune system will foster the will lead to the development of more effective therapies for metastatic renal cell cancer.

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