Cytokines in the Management of Advanced Renal Cell Cancer

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Contents

Key Points

- IFN-α has modest activity in metastatic renal cell cancer (mRCC). Currently, its main therapeutic role is in combination with bevacizumab, which has been approved for first-line therapy.
- High-dose IL-2 can lead to durable responses not seen with any other drug, but should be considered as first-line therapy only for highly selected favorable or intermediate-risk patients due to its severe systemic toxicities.
- Proper management of adverse events due to high-dose IL-2 can limit toxicity and improve patient outcomes.
- Combinations of immunotherapy and cytotoxic chemotherapy are not effective and therefore not recommended for current treatment of mRCC.
- Combination of immunotherapy and biologic agents is of limited use due to increased toxicity, with the exception of IFN and bevacizumab, which appears to be both tolerable and efficacious.
- Efforts are underway to elucidate molecular markers that will help predict benefit from the administration of high-dose IL-2.

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Table 15.1 Selected immune-based approaches

Interferons (*INF*): interferon-α, interferon-β, interferon-γ
Interleukins (*IL*): interleukin-2, interleukin-12, interleukin-21
Cytokine combination strategies
Cytokine combinations
Cytokines + cellular therapies (e.g., IL-2 and tumor-infiltrating lymphocytes)
Cytokines and chemotherapy or biologics (e.g., INF + bevacizumab)
Mini-allogeneic transplant approach
Reduced-intensity conditioning therapy followed by circulating hematopoietic progenitor cell transplantation
Tumor vaccines
Tumor cell-based vaccines
Gene-modified tumor cell vaccines
Dendritic cell-based vaccines
Heat shock protein-based vaccine
Antigenic peptide-based vaccines
Immune checkpoint inhibition
Anti-PD1 monoclonal antibodies (nivolumab)
Inhibition of cytotoxic T-lymphocyte-associated antigen 4 (CTLA4)

15.1 Overview

The hypothesis that renal cell cancer (RCC) may be sensitive to immunologic manipulation initially came from the rare but fascinating phenomenon of spontaneous tumor regression in RCC patients [1]. The mechanism for spontaneous regression is unclear, although immunologic factors have been implicated. This has led to the evaluation of immune-based strategies in the management of advanced disease. Table 15.1 provides a summary of selected immune-based approaches [2] that have been employed in RCC; however, this chapter will focus predominantly on cytokine-based therapies (see Chaps. 14 and 16 for a discussion of other immune-based therapies, including vaccines and checkpoint inhibitors).

Cytokines are non-antibody proteins used for cellular communication and can act as mediators or regulators of the immune system. Some of the most studied cytokines include interferon alpha (IFN-α) and interleukin-2 (IL-2). These cytokines have long been considered important factors in the activation and development of an immune response, including responses against tumor cells. These responses are believed to be mediated through enhanced T-cell, dendritic cell, and natural killer (NK)-cell activity directed against antigenic RCC cells. The discovery of methods to manufacture and purify cytokines through recombinant technology triggered a series of trials testing these agents in patients with advanced RCC.

15.2 Interferon

IFN-α is a cytokine that stimulates cytolytic activity and proliferation of NK cells, phagocytic functions and production of other cytokines by macrophages, and the expression of MHC molecules in most immune cells [3]. Another mechanism by which IFN-α operates is through regulation and proliferation of cytotoxic CD8+ T cells [4]. It is thought that IFN-α stimulates the proliferation, activation, and generation of CD8+ T cells leading to tumor cell destruction. In cancer, there is also dysregulation observed between T-helper (Th) 1 and Th2 CD4+cells, characterized by an imbalance in Th2 CD4+ cell production [5]. Th1 CD4+ cells mature to become macrophage-activating cells, whereas Th2 CD4+ cells turn into B cells. IFN-α can stimulate the expression of IL-12 receptors on Th1 cells leading to selective promotion of the Th1 response and also causing a suppression of IL-4 and IL-13 gene expression. This culminates in a subsequent dampening of the Th2 response [6]. This series of events is believed to lead to an enhancement in the activity of the cellular immune response wherein monocytes and macrophages exert a direct negative effect on tumor cell growth and proliferation via their phagocytic mechanisms. IFN-α also exerts its antitumor activity through its ability to upregulate MHC gene expression in tumor cells. Most tumor cells exhibit a partial or complete loss of MHC antigens on the cell surface [7]. This does not allow for dendritic cells – antigen-presenting cells (APCs) that are potent stimulators of IFN-α production – to recognize

nonself antigens and to initiate the cytokine cascade. This can then lead to an indirect enhancement of the proliferation of tumor cells. Antitumor therapies that upregulate MHC gene expression in tumor cells, such as IFN-α, are thought to induce immunologic rejection of the tumor cells through the activation of APCs and cell-mediated cytotoxicity.

Three categories of interferons of relevance to RCC have been described: IFN-α, IFN-β, and IFN-γ. These IFN species vary according to the usual cell of derivation. IFN-α is mainly derived from white blood cells and IFN-β from fibroblasts, while IFN-γ is typically derived from T cells. As noted earlier, recombinant technology has allowed for the efficient manufacture of these molecules for human testing in clinical trials. The most active agent appears to be IFN-α, while IFN-β and IFN-γ appear to be of limited clinical utility. For example, in a phase II trial single-agent IFN-β serine in RCC, there was no signal of enhanced efficacy for IFN-β serine compared to historical data with IFN-α [8]. Furthermore, a placebo-controlled trial in metastatic RCC of IFN-γ 1b (dosed at 60 μg per square meter of body surface area subcutaneously once weekly) showed no significant differences between the groups in terms of response rates, time to disease progression, or overall survival. Thus, further clinical development of IFN-β and IFN-γ had been halted, while IFN-α was subsequently evaluated in a series of clinical trials.

Recently, there has been revival of interest in IFN-γ research. A study by Chen et al. draws attention to two issues limiting IFN-γ efficacy, which include previously exploiting only its immunomudulatory properties rather than its direct tumoricidal properties and its poor pharmacokinetics, which was improved by developing an antibody-cytokine conjugate. In this in vitro study, the investigators demonstrate that both human and murine IFN-y fused to an anti-CD70 antibody are able to induce RIP1-dependent necrosis in RCC cells in the presence of the proteasome inhibitor bortezomib [9]. Further studies evaluating IFN-γ are ongoing.

Wide ranges of dosing regimens and schedules for IFN-α have been employed across clinical trials. At this time, no one dose schedule has been definitively identified as the most optimal. A regimen of nine million units given by subcutaneous injection, three times a week for 12 weeks or to disease progression, has been widely used in the control arms of recently completed randomized phase III trials [10–14]. In 1990, IFN-α was approved for the treatment of metastatic renal cell carcinoma in Western Europe based on nonrandomized phase II studies. Notably, IFN-α has never received US Food and Drug Administration (FDA) approval for its use in advanced RCC (Fig. 15.1 shows the proposed 3D structure for the recombinant IFN-α2b molecule as depicted in RCSB Protein Data Bank at http://www.rcsb.org).

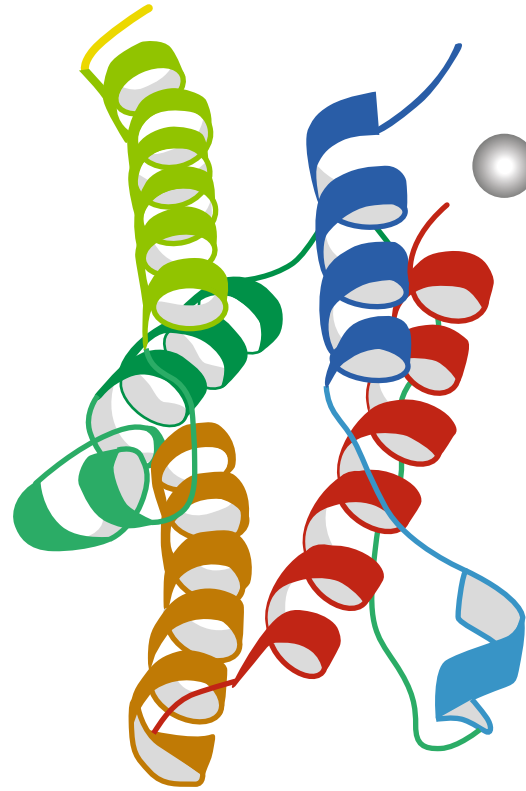

Fig. 15.1 Proposed three-dimensional structure of recombinant interferon alpha-2b (http://www.rcsb.org)

A number of randomized phase III studies have been completed using IFN-α in the setting of metastatic RCC; it must be noted that none of the trials were placebo controlled. One study

compared IFN-α2b with medroxyprogesterone acetate (MPA) [15, 16]. Patients with mRCC were randomized to receive either subcutaneous IFN-α2b (three doses, five million units, five million units, and ten million units for the first week, and then ten million units three times per week for a further 11 weeks, with a total number of patients = 174) or oral MPA (300 mg once daily for 12 weeks, with a total number of patients = 176). A total of 111 patients died in the IFN-α2b group compared to 125 patients in the MPA group. There was a relative reduction in the risk of death by 28 % in the IFN-α group (hazard ratio 0.72 [95 % CI 0·55–0·94], $p = 0 \cdot 017$). IFN-α2b gave an absolute improvement in 1-year survival of 12 % (MPA 31 % survival vs. IFN-α2b 43 %) and an improvement in median survival of 2.5 months (MPA 6 months vs. IFN-α2b 8.5 months). Side effects were more common with the IFN-α2b group and included moderate to severe lack of appetite, nausea, lack of energy, shivering, and dry mouth. Other studies compared IFN-α2a plus vinblastine with either vinblastine alone [16–18] or against MPA [19]. When IFN-α and vinblastine were compared to vinblastine alone, the interferon-containing arm was superior in terms of response rates (17 % vs. 3 %) and survival (67.6 vs. 37.8 weeks, $p < 0.05$). On the other hand, when the combination IFN-α2a and MPA was compared to MPA alone, there was a significant difference in response rate (21 % vs. 0 %), but not in overall survival (16 months vs. 10 months, $p = 0.19$).

This notion was confirmed in a 2005 Cochrane review of published randomized controlled trials employing IFN-α in advanced RCC [20]. Pooled results from four trials consisting of 644 patients suggested that IFN-α was superior to controls (odds ratio for death at 1 year was 0.56, 95 % CI 0.40–0.77), while the overall hazard ratio for death was 0.74 (95 % CI, 0.63–0.88). The pooled remission rate was 12.5 % for IFN-α versus 1.5 % for controls, with a pooled odds ratio of 7.6 (95 % CI 3.0–19.2). The weighted average improvement in survival was 3.8 months (11.4 vs. 7.6 months). Based on these results, IFN-α became a reasonable community standard for the systemic management of advanced RCC. Recently, the discovery of novel targeted agents has decreased the use of IFN-α with its application limited to combination therapy with biologic agents (discussed later in this chapter and in Chap. 17).

Observational case reports noted improved responses and survival when the primary tumor was removed surgically. This was the impetus for a randomized trial comparing IFN-α to nephrectomy followed by IFN-α in mRCC conducted by the Southwest Oncology Group (SWOG trial 8949). The results were noteworthy for a significant improvement in median overall survival in patients who had a nephrectomy prior to immunotherapy. The median overall survival in the group receiving IFN-α only was 8.1 months, while the median overall survival in the group of patients who received a nephrectomy followed by IFN-α was 11.1 months [21]. An updated analysis with a median follow-up of 9 years was conducted to evaluate predictors of overall survival. Patients randomized to nephrectomy continued to demonstrate improved overall survival (HR 0.74, 95 % CI 0.57–0.96, $p = 0.022$). Multivariate analysis showed that performance status 1 vs. 0 (HR 1.95, $p < 0.0001$), high alkaline phosphatase (HR 1.5, $p = 0.002$), and lung metastasis only (HR 0.73, $p = 0.028$) were overall survival predictors [22]. The findings seen in the SWOG 8949 were confirmed by another similar but much smaller randomized trial conducted by the European Organization for Research and Treatment of Cancer Genitourinary Group (EORTC 30947). This trial reported a significant increase in the time to progression (5 months vs. 3 months) and median survival duration (17 months vs. 7 months) in the group that underwent debulking nephrectomy followed by IFN-α when compared to IFN-α alone [23]. Furthermore, when both of these trials were combined in a meta-analysis conducted by the Cancer Care Ontario Program in Evidence-Based Care (CCO-PEBC), the overall median survival time for patients treated with nephrectomy and INF-α2b was 13.6 months compared with 7.8 months for patients treated with INF-α2b alone ($p = 0.002$). This represents a 31 % decrease in the risk of death in the surgical arm [24].

These data support the role for cytoreductive nephrectomy. Among the many caveats here are that some patients who undergo surgery may have resultant complications that either delay or make them ineligible to receive further systemic therapy. Nevertheless, IFN-α following debulking nephrectomy in patients fit enough to undergo the procedure should be considered as part of the standard treatment strategy in mRCC.

15.3 Interleukin-2

Interleukin-2 is an immune cytokine that is essential in the activation of a specific response to antigens by T cells, as well as crucial in triggering the innate immunity by stimulating several functions of NK cells and macrophages [25]. The actual mechanism by which IL-2 exerts its antitumor effects is unknown, but there are several hypotheses. Experiments in animal models showed that IL-2 can offset defective antigen recognition and overcome tolerance, thus suggesting its use as therapy to stimulate tumor destruction by T- or NK-cell activation while overcoming possible forms of tolerance or immunological ignorance which are known to occur toward tumor antigens [25]. In vitro studies with murine and human cells showed that IL-2 can activate lymphokine-activated killer (LAK) cells, a subpopulation of lymphocyte effectors that include NK, T, and NKT cells. These cells are endowed with the capacity of killing neoplastic cells in a MHC-unrestricted fashion. Clinical trials have noted a response in the tumor burden of patients treated with IL-2, but the mechanism of such clinical responses has not been clarified since accumulation of LAK cells in metastatic deposits (i.e., direct tumor kill) has not yet been demonstrated [25]. Thus, tumor shrinkage has been attributed to nonspecific cytotoxic activity of LAKs as well as to activation of tumor-specific T cells, but the release of tumor cytotoxic cytokines (e.g., TNF-α) by activated lymphocytes may also have contributed.

A total of 255 patients with metastatic RCC were entered onto seven phase II clinical trials and treated with high-dose IL-2 at either 600,000 or 720,000 international units per kg (IU/Kg) per dose intravenously every 8 h for up to 14 consecutive doses until maximally tolerated [26, 27]. A second identical cycle of treatment was scheduled following 5–9 days of rest. These courses could be repeated every 6–12 weeks in stable or responding patients for a total of three courses. The overall response rate was 14 % with 12 (5 %) complete responses and 24 (9 %) partial responses. The median response duration was 19 months for partial responders and had not been reached for complete responders. The median overall survival was 16.3 months [27]. These studies showed that patients who responded to IL-2 could attain a durable response and were living longer than historical controls that had received no therapy. The durability of response was confirmed elsewhere when 6 % of patients with metastatic renal cell cancer treated with high-dose IL-2 were found to be in complete remission from 4 to 10 years after treatment [28]. Based on the phase II single-arm studies discussed above, the FDA approved the dose of 600,000 IU/kg (high-dose IL-2) in 1992 for the treatment of metastatic RCC as front-line therapy.

High-dose IL-2 is associated with systemic toxicities and can affect every organ system in the body. Patients are generally admitted to an intensive care unit or similarly staffed unit for the administration of this drug. Prior to initiating therapy, one must make sure that the patient does not have significant cardiac, pulmonary, or renal disease. During a typical treatment course, patients will often experience the following symptoms occurring at different time points within the course. Within 2–3 h after the first or second dose of IL-2, patients often start experiencing fevers and chills. Around this same time, patients will also start experiencing mild hypotension and tachycardia that will progressively become more severe with each dose. They will typically establish a new baseline blood pressure around 20–30 mmHg below their usual blood pressure. Oliguria usually starts within the first 24 h, requiring small boluses of fluid to keep urine output greater than 20 ml/h. As the patient nears the end of the cycle, hypotension and

oliguria can become refractory to judicious hydration (no more than 1–1.5 L per day) requiring pharmacologic intervention including dopamine and phenylephrine. Pulmonary congestion, increase in weight, and peripheral edema may then ensue due to fluid overload and as a manifestation of capillary leak. Nausea, vomiting, and diarrhea also occur closer to the completion of the cycle [29]. Neurologic, infectious, metabolic, and dermatologic effects can also be manifested; these are specified in more detail in Table 15.2. These symptoms are primarily thought to be due to capillary leak syndrome (CLS) and lymphoid infiltration within the organ systems. Proper management of the adverse events discussed above can limit toxicity and improve patient outcomes.

Given the difficulty of administering high-dose IL-2, attempts were made to find a lower dose of IL-2 or an alternative administration schedule, whereby its antitumor activity would be preserved with diminished or mitigated side effects. A three-arm study sponsored by the National Cancer Institute compared high-dose IL-2 administered at 720,000 international units/kg to low-dose IL-2 dosed at 72,000

Table 15.2 Side effects and management of high-dose IL-2 administration

System	Adverse reaction	Treatment
Cardiovascular	Hypotension due to capillary leak syndrome	Fluids (normal saline), limit to 1–1.5 L/day
		Add phenylephrine drip if refractory to fluids
	Sinus tachycardia due to hypotension	Increase time between doses of IL-2
	Atrial fibrillation or ventricular arrhythmia	Hold IL-2, evaluate for ventricular damage (ischemia), correct electrolytes and anemia, and use medications as needed. Restart IL-2 only if arrhythmia is easily corrected
	Peripheral edema	Hold IL-2, watchful waiting as this will resolve over time or with the use of diuretics. Elevate extremity
	Increased troponin or creatinine kinase	Hold IL-2; exercise ECHO before next dose of IL-2 to evaluate for myocardial dysfunction. If evidence of ischemia, stop IL-2
Pulmonary	Hypoxia – fluid overload	Diuretics
	Tachypnea – due to hypoxia or metabolic acidosis	Diuretics if due to fluid overload
		IV sodium bicarbonate
Renal	Elevated creatinine with adequate urine output	Fluids (normal saline), limit to 1–1.5 L/day
		Add dopamine drip if unresponsive/unable to tolerate fluids
		If oliguria and/or elevated SCr, hold IL-2
Neurologic	Confusion, disorientation, hallucinations	Hold IL-2 until resolution; then rechallenge. If symptoms are recurrent, then hold treatment
Metabolic	Metabolic acidosis	Bicarbonate infusion (100 meq/L) to keep serum bicarbonate level >18 meq/L
	Hypokalemia	Replace electrolytes as needed
	Hypocalcemia	
	Hypomagnesemia	
Systemic	Fevers and chills	Premedication with acetaminophen 650 mg po q4h and indomethacin 25 mg po q6h. An H2 blocker to protect the gastric mucosa should be utilized. Consider infectious etiology if first fever is over 24 h after therapy initiation
	Rigors	Meperidine 25–50 mg IV × 1
	Nausea and vomiting	Ondansetron 4 mg IV × 1
		Prochlorperazine 25 mg IV × 1
Skin	Dermatitis	Topical emollients and antihistamines. Avoid steroid- or alcohol-containing lotions
	Pruritus	Histamine antagonist (e.g., diphenhydramine)
Gastrointestinal	Diarrhea	Diphenoxylate or loperamide as needed

international units/kg to low-dose subcutaneous daily IL-2 [30]. Response rate was significantly higher with the high-dose compared with the low-dose IV and subcutaneous schedules (21 % vs. 13 % vs. 10 %, respectively). There were more adverse events in the high-dose IV therapy group, but no deaths were attributed to it. There was also a trend toward more durable responses with the high-dose IL-2 group. Overall, there was no difference in overall survival. Toxicities though were seen much less frequently in the low-dose arm, especially the major side effect of hypotension. Although, subcutaneous IL-2 did not have a significant response rate in this study, impressive response rates were seen in patients with metastatic RCC in other phase II trials [31–33]. This led to the popularization of this mode of therapy in European countries in the 1990s. There was however no definitive studies conducted to fully evaluate its utility and its place among the treatment options for metastatic RCC.

More recently, a systematic review evaluating patients with unresectable or mRCC, comparing treatment regimens containing IL-2 to those without, revealed that mortality at 1 year was not statistically significant between IL-2-based regimens and non-IL-2 controls [34]. The pooled response rates, however, were higher in patients receiving IL-2-based regimens (range, 9–39 %) compared with non-IL-2 controls (0–20 %). There was an increase in toxicity in the IL-2-based regimens compared to non-IL-2 controls; however, most patients tolerated treatment well. Of note, this review did not include any high-dose IL-2 trials, as there are no known randomized trials comparing high-dose IL-2 to non-IL-2 control or placebo (all prior studies were phase II single-arm studies).

Based on the data above, non-high-dose IL-2-containing regimens do not appear to provide superior treatment efficacy over non-IL-2-containing regimens and are associated with increased toxicity. High-dose IL-2 does provide higher response rates, albeit with higher toxicity, and can provide a small chance for a complete and durable remission and hence continues to play a role in the treatment of mRCC in the appropriate treatment population.

15.4 Interferon plus Interleukin-2 Combination(s)

Interferon alpha and interleukin-2 have been shown to have efficacy in the treatment of metastatic RCC; however, whether these two drugs given in combination would be more efficacious was the subject of intense investigation in the 1990s.

Phase II trials were first performed to assess combining these two agents in hopes of a synergistic response. One study evaluated high-dose IL-2 alone (1.33 mg/m^2; approx. 600,000 IU/kg) versus non-high-dose IL-2 (0.8 mg/m^2) in combination with IFN-α in patients with mRCC [35]. In this study, patients in both arms had responses to therapy, but the IL-2 alone arm (high-dose IL-2) was noted to have a higher objective and durable response rate. This study concluded that IL-2 alone, when given as a high-dose IV bolus, was active in metastatic RCC and that combining it with IFN-α was not as efficacious. A somewhat varying conclusion was noted from a publication around the same time that had tested alternate daily dosing of intravenous IL-2 and subcutaneous IFN-α [36]. In that study, 36 patients received 14 days of daily alternating treatments of IL-2 and IFN-α every 6 weeks for up to four cycles. Of the 30 patients who completed at least two cycles, there were nine objective responses, and seven of them had relapse-free survival times that were >6 months, the longest being 2 years. The toxicity was reported to be less, and these results led to a conclusion that the combination of IL-2 and IFN-α was active, rivaled responses of each agent alone from other phase I and II studies, and warranted further study. Other phase II studies were carried out in order to evaluate the use of subcutaneous IL-2 and IFN-α [37–39]. These studies noted encouraging responses with less toxicity, but results were discordant and did not provide definitive conclusions.

In this setting, the Groupe Francais d'Immunotherapie initiated one of the first randomized phase III studies that established the efficacy of IFN-α and IL-2 in patients with metastatic RCC in 1998 [40]. Patients were randomized to receive either subcutaneous injections of

IFN-α, continuous intravenous infusion of IL-2, or both given in combination. The dose of IL-2 used in this study was an intermediate one, 18,000,000 IU/m^2 per day (i.e., non-high dose). Response rates were 6.5 %, 7.5 %, and 18.6 % ($p=0.01$) for the groups receiving IL-2, IFN-α, and IL-2 plus IFN-α, respectively. Over a period of 1 year, the event-free survival was 15 %, 12 %, and 20 %, respectively ($p=0.01$). Despite the encouraging results of combined therapy, there was no difference in overall survival between the three groups. The investigators also noted more adverse events in the combined immunotherapy group. Hence, it could not be concluded that combined therapy provided a significant advantage. Another phase III study evaluated the inpatient administration of high-dose IL-2 to the outpatient regimen of subcutaneous IL-2 and IFN-α [41]. The response rate was 23.2 % for high-dose IL-2 versus 9.9 % for IL-2 and IFN-α ($p=0.018$). Ten patients receiving high-dose IL-2 were progression-free at 3 years versus three patients receiving IL-2 and IFN-α ($p=0.082$). These results suggest that high-dose IL-2 is more efficacious when compared to outpatient subcutaneous IL-2 and IFN-α combined.

In summary, there were a variety of combinations of IL-2 and IFN-α that were tested in the 1990s and early 2000s. Overall, the combination appeared to have some efficacy, but randomized phase III trials did not demonstrate an improved survival rate when comparing varying doses of IL-2 combined with IFN-α to that of high-dose IL-2 alone. Hence, high-dose IL-2 alone should remain a standard of care option for highly selected patients with mRCC.

15.5 Cytokines in Combination with Chemotherapy

There were subsequent efforts to improve upon the modest survival advantage seen with IFN-α. However, when combinations with cytotoxic or differentiating drugs were attempted, the results were disappointing. For instance, the differentiating agent 13-cis retinoic acid showed some promise in the treatment of metastatic RCC, but when this drug was combined with IFN-α, the results showed no improvement in survival when compared to monotherapy with IFN-α [42]. Vinblastine was considered to be somewhat promising when phase II studies showed response rates varying from 16 to 39 % [43]. Unfortunately, phase III trials that compared the combination of IFN-α with vinblastine did not show any improvement in overall survival when assessing it against IFN-α alone [17]. When IFN-α and vinblastine were compared to medroxyprogesterone acetate, which is essentially a placebo arm, no difference in overall survival was noted [18]. In that study, the response rate was 20.5 % in the combination therapy arm and 0 % in the control arm. The lack of a significant difference in survival may have been due to the small number of patients in the study (89 patients total), due to an increase in toxicities in the combination therapy arm, or because response rates in this case do not correlate well with overall survival. Similar results were again noted when the combination of IFN-α and vinblastine showed inferior results in a large phase III trial that compared this combination to an arm with subcutaneous IL-2 and subcutaneous IFN-α and 5-fluorouracil or oral 13-cis-retinoic acid [44].

The fluoropyrimidine 5-fluorouracil had been tested in phase II trials in patients with metastatic renal cell cancer, and response rates varied from 12 to 39 % [45, 46]. 5-Fluorouracil looked to be fairly promising when added to immunotherapy; however, a direct phase III comparison between cytokines plus 5-fluorouracil versus immunotherapy alone was required. This was fulfilled with the completion of the phase III MRC RE04/EORTC GU 30012 randomized study [47]. In that trial, 1,006 treatment-naive RCC patients were randomly assigned to receive interferon alpha-2a alone or combination therapy with interferon alpha-2a, interleukin-2, and fluorouracil. The primary endpoint was overall survival. Serious adverse events were comparable between the arms. At a median follow-up time of 37 months, median overall survival time was reported to be 18.8 months for patients receiving interferon alpha-2a versus 18.6 months for those receiving combination therapy. The hazard ratio

for overall survival was 1.05 [95 % CI 0.90–1.21, $p=0.55$], and the absolute difference was 0.3 % (−5.1 to 5.6) at 1 year and 2.7 % (−8.2 to 2.9) at 3 years. This large randomized trial clearly demonstrated that the polypharmacy approach of cytokines plus cytotoxic chemotherapy was no more efficacious than cytokines alone.

15.6 Cytokines in Combination with Biologic Agents

Over the next decade, the emergence of molecular targeted therapy with tyrosine kinase inhibitors (TKIs) and the mammalian target of rapamycin (mTOR) inhibitors supplanted the use of IFN-α and IL-2. These new drugs (including sunitinib and temsirolimus, both of which are discussed in greater detail elsewhere in this textbook) were more efficacious than single-agent IFN-α in randomized studies. Overall, these studies have shown that combined therapy leads to greater toxicity, which limits their use as a chronic treatment option. Unlike the agents discussed above, bevacizumab, a monoclonal antibody that binds to and neutralizes vascular endothelial growth factor (VEGF), appears to be both tolerable and efficacious in combination with IFN.

In the AVOREN trial [9] which was principally conducted in Europe, 649 patients with previously untreated metastatic RCC were randomly assigned to receive bevacizumab (10 mg/kg every 2 weeks) plus IFN-α (nine million international units subcutaneously three times a week; $n=327$) or IFN-α plus placebo ($n=322$). The progression-free survival was found to be 10.2 months with bevacizumab plus IFN-α versus 5.4 months with IFN-α plus placebo, corresponding to a hazard ratio [HR] of 0.63 ($p<0.001$). The overall response rate (ORR) was also improved in the combined therapy arm (30.6 % versus 12.4 %; $p<0.001$). There was a trend toward overall survival (OS) improvement, with the median overall survival time of 23.3 months with bevacizumab plus IFN-α versus 21.3 months with IFN-α plus placebo (unstratified hazard ratio [HR] = 0.91; 95 % CI, 0.76–1.10; $p=0.3360$; stratified HR = 0.86; 95 % CI, 0.72–1.04; $p=0.1291$). The main confounder was that >50 % of patients in both arms received at least one other post-protocol therapy, including very active tyrosine kinase inhibitors. The above findings were confirmed in additional trials discussed below.

The Cancer and Leukemia Group B (CALGB) 90206 trial was an open-label, phase III trial conducted in the United States, comparing bevacizumab plus IFN-α to IFN-α monotherapy in 732 previously untreated mRCC patients [12]. The median PFS was 8.5 months in patients receiving bevacizumab plus IFN-α compared to 5.2 months in patients receiving IFN-α monotherapy (log-rank $p<0.0001$). The ORR was also improved in the combined therapy arm (25.5 % versus 13.1 %, respectively; $p<0.0001$). The median OS was 18.3 months for bevacizumab plus IFN vs. 17.4 months for IFN monotherapy (unstratified log-rank $p=0.097$; stratified HR = 0.86; 95 % CI, 0.73–1.01; stratified log-rank $p=0.069$). OS favored the bevacizumab plus IFN arm; however, it failed to meet significance, which may be due to postprogression therapy, a factor that was not anticipated when the trial was designed [13].

The TORAVA trial was an open-label, phase II trial conducted in France ($n=171$), comparing the combination of bevacizumab (10 mg/kg every 2 weeks) and temsirolimus (25 mg weekly; group A) versus sunitinib (50 mg/day for 4 weeks followed by 2 weeks off; group B) or the combination of IFN-α (9 mIU three times per week) and bevacizumab (10 mg/kg every 2 weeks; group C). The median PFS was 8.2 months (95 % CI 7.0–9.6) in group A, 8.2 months (5.5–11.7) in group B, and 16.8 months (6.0–26.0) in group C. Grade ≥3 AEs were reported in 77, 60, and 70 % of patients in groups A, B, and C, respectively. The authors concluded that the toxicity of temsirolimus and bevacizumab was much higher than anticipated and clinical activity was low compared to the benefit expected from sequential use of each targeted therapy, hence not recommended for first-line treatment in patients with mRCC. The combination of IFN and bevacizumab achieved favorable PFS results [48].

The Bevacizumab and Low-Dose Interferon (BEVLiN) trial was a single-arm, phase II trial

($n = 146$) evaluating the combination of bevacizumab (10 mg/kg every two weeks) and low-dose IFN (3 MIU three times weekly) in patients with untreated mRCC in order to determine if the use of low-dose IFN can maintain clinical benefit while reducing toxicity. The median PFS and OS were 15.3 months (95 % CI, 11.7–18) and 30.7 months (95 % CI, 25.7 – not reached), respectively. The overall response rate (ORR) was 28.8 % (95 % CI 21.4–37.1). Any-grade and grade ≥3 IFN-associated adverse events occurred in 53.4 % and 10.3 % of patients, respectively, and were lower by 17 % and 18 %, respectively, compared with the AVOREN subgroup. The authors concluded that compared with the historical control AVOREN subgroup, low-dose IFN with bevacizumab resulted in a more favorable safety profile, with similar efficacy [49].

15.7 Predictive Clinical Features and Biomarkers for the Use of Cytokines to Treat mRCC

There are a multitude of different agents now available for the treatment of mRCC, yet there are only limited data on how best to determine which patient population cytokines will be most effective, especially given the low response rates and substantial side effects of such therapies. Recent advances in the understanding of the molecular mechanisms underlying RCC are vital for establishing the optimal treatment strategies in patients with mRCC with a drive toward personalized medicine. Here, selected results of recent research into potential biomarkers related to cytokines are discussed.

Retrospective studies evaluated clinical features and/or molecular markers to assess if these could be used to predict response to therapy. Clinical features that were identified included clear cell histology [50] as well as a favorable score on the UCLA Survival after Nephrectomy and Immunotherapy (SANI) scale [51]. The SANI score was developed as an algorithm capable of predicting survival in patients with metastatic RCC who underwent nephrectomy and received IL-2-based immunotherapy. The primary endpoint was survival and was assessed based on clinical, surgical, and pathological features. The multivariate analysis showed that the presence of lymph node involvement, constitutional symptoms, multiple metastatic sites (as compared to bone- or lung-only metastases), sarcomatoid histology, and elevated TSH level had adverse effects on survival.

Upton et al. examined the specimens from patients with RCC treated with IL-2 to identify histologic features that predict response. They found that for clear cell carcinomas, response to IL-2 was associated with the presence of alveolar features and the absence of papillary and granular features [50].

In addition to clear cell histology and the SANI score, the enzyme carbonic anhydrase-IX (CA-IX) has been identified as a potential biomarker to predict outcomes in patients with RCC. It was found to be expressed in 94 % of clear cell RCC tumors but absent in most normal tissue. Low CA-IX staining (<85 %) of tissue microarrays by immunohistochemistry was a poor prognostic factor for survival for patients with mRCC, with a hazard ratio of 3.10 ($p < 0.001$) [52]. A subsequent case-control study by Atkins et al. showed an association between higher levels of CA-IX expression and response to IL-2. The response to IL-2 was further improved in those patients with high CA-IX expression level and histologic predictors based on the Upton pathology model [53]. There was an attempt to prospectively validate these features in a clinical trial of patients with mRCC treated with high-dose IL-2. Preliminary results of this study (SELECT trial) showed that clear cell histology might be the salient clinical feature that selects patients who respond to IL-2 [54]. Unfortunately, it failed to show the predictive capacity of either the CA-IX expression or the favorable histologic features as reported in prior studies.

Recently, data were presented evaluating PDL1 or PDL3 (programmed death ligand 1 or 3) expression and their association with response to initial therapy with IL-2 or subsequent therapy (VEGFR TKI). In the 17 patients whose tumors were positive for both PDL1 and PDL3, the

overall response rate (ORR) to IL-2 was 52.9 %. In the 27 patients that were negative for PDL1 and PDL3, the ORR to IL-2 was 11.1 %. With regard to subsequent VEGFR TKI therapy, those patients whose tumors were positive for PDL1 and PDL3 expression had a shorter duration of VEGFR TKI therapy compared to those that were negative (9.0 months vs. 42.5 months, respectively) [55].

The treatment of mRCC with both IL-2 and IFN-α relies on the ability to activate CD4+ and CD8+ T cells. B7-H4 is a B7 member identified as an inhibitory modulator of the T-cell response, and the upregulation of this ligand is thought to lead to immune escape in mRCC. Krambeck et al. have shown that aberrant RCC expression of B7-H1 leads to disease progression and decreased survival. Furthermore, those tumors expressing both B7-H1 and B7-H4 are at an even greater risk of death from RCC. Because it appears that both of these ligands impair T-cell function, this group infers that they may be useful in determining which patients may respond to IL-2 therapy [56]. Xu et al. confirmed these findings where B7-H4 expression was seen in 59 % of tumor specimens collected from RCC patients undergoing radical nephrectomy. Exposure of a clear cell RCC (ccRCC) cell line to IL-2, IFN-α, and IFN-γ leads to increased expression of both protein and mRNA of B7-H4 and was most apparent after exposure to IFN-γ. Masking of B7-H4 with a specific blocking antibody increased the T-cell-mediated killing of the ccRCC cells. These observations may present evidence for the role of B7-H4 in tumor immune escape in mRCC and may be the reason for the low efficacy of IL-2 and IFN-α and inability to observe efficacy of IFN-γ. In addition, B7-H4 may be further studied as a potential biomarker [57].

Although single-agent IFN-α is rarely used in many resource-rich nations, its use continues in many parts of the world. Due to the low response rate seen with single-agent IFN-α, identification of potential predictive markers of response is necessary to determine which subset of patients will benefit from this drug. Recently, Eto et al. has analyzed a large number of genomic polymorphisms from DNA extracted from whole blood of RCC patients. In an initial retrospective study, they evaluated 463 SNPs on 33 candidate genes and found that SNPs in the signal transducer and activator of transcription 3 gene (STAT3) were associated with a better response to IFN-α in patients with mRCC. In a follow-up trial, these investigators evaluated the correlation between the antitumor effects of IFN-α and 11 SNPs. Overall response (CR and PR) to IFN-α was found not to be associated with any of the 11 SNPs (including STAT3). However, when assessing the clinical response defined as CR, PR, and stable disease of >24 weeks, a significant association was observed between the STAT3-2 and clinical response to IFN-α ($p=0.039$). Furthermore, the C/C genotype of STAT3-2 was associated with the clinical response of IFN-α and the secondary endpoint of overall survival. Note that this study was completed in the Japanese population only and generalization of results to other races/ethnicities is uncertain [58].

At this time, clear clinical predictive factors or molecular biomarkers for the benefit of IL-2 or IFN-α remain elusive and are not yet ready to adopt into clinical practice, but are the focus of ongoing research.

Clinical Vignette

A 50-year-old male with no past medical history noted a cough that has been troubling him for the last 4 weeks. He tried a number of over-the-counter cough suppressants with only minimal improvement in his symptoms. His primary care physician ordered a chest radiograph that revealed numerous lung nodules, the largest being 2×2 cm in the left lower lobe. Follow-up CT scans of his chest, abdomen, and pelvis were then performed and confirmed the lung nodules as well as a 7 cm mass in his right kidney. A biopsy of the left lower lobe lung nodule was performed, and the pathology was consistent with carcinoma with clear cells, establishing the diagnosis of

metastatic renal cell carcinoma. He underwent a cytoreductive nephrectomy, confirming the diagnosis of renal cell cancer. This patient is otherwise healthy and asymptomatic; he runs 3 miles a day and is taking no medications. Although there are many therapeutic choices, high-dose intravenous interleukin-2 should be strongly considered for this young, healthy patient with limited metastatic disease confined to the lungs, as there is potential for a durable complete response. In the 1990s, the mainstay of therapy for metastatic renal cell carcinoma included the use of cytokine agents. Even with the discovery of potent, efficacious, and less toxic biologic agents, there is still a limited role for the use of cytokines today. This chapter will discuss the history and past achievements of cytokine-based immunotherapy as well as the future of these agents.

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