Now in its 19th Year

Kidney Cancer JOURNAL

Official Journal of The Kidney Cancer Association

Volume 19
Number 2
2021

www.Kidney-Cancer-Journal.com

Resource-Efficient Pooled Sequencing Expands Translational Impact

Renal Cell Carcinoma Associated with Germline Mutations in the Krebs Cycle

Changes, Challenges & Opportunities in Cancer Care During COVID-19 Era & Beyond

An Educational Service for Medical Oncologists, Hematologist-Oncologists, and Urologists

Important Safety Information

Because of the severe adverse events which generally accompany Proleukin therapy at the recommended dosages, a thorough clinical evaluation should be performed to identify patients with significant cardiac, pulmonary, renal, hepatic, or CNS impairment in whom Proleukin is contraindicated. Patients with normal cardiovascular, pulmonary, hepatic, and CNS function may experience serious, life-threatening or fatal adverse events. Adverse events are frequent, often serious, and sometimes fatal. The following adverse events (Grades 1-4) were seen in ≥ 30% of 525 patients (255 with metastatic renal cell cancer and 270 with metastatic melanoma) treated with Proleukin: hypotension (71%), diarrhea (67%), oliguria (63%), chills (52%), vomiting (50%), dyspnea (43%), rash (42%), bilirubinemia (40%), thrombocytopenia (37%), nausea (35%), confusion (34%), and creatinine increase (33%).

Please see accompanying full Prescribing Information and complete Boxed Warning on the following pages. You are encouraged to report negative side effects of prescription drugs to the FDA. Visit www.fda.gov/medwatch or call 1-800-FDA-1088.

Proleukin® (aldesleukin) is indicated for the treatment of adults with metastatic renal cell carcinoma (mRCC) and metastatic melanoma (mM).

20 extraordinary years of laugh lines[1-4]

With Proleukin, a life without metastatic cancer may be possible beyond 20 years for some mRCC patients and beyond 15 years for some mM patients[1-4]

Proleukin is the only immunotherapy that offers some patients the proven possibility to achieve a durable complete response for over 2 decades—and counting.[1-4] Objective response was seen in 16% of patients with mM (6% had a complete response and 10% had a partial response) and in 15% of patients with mRCC (7% had a complete response and 8% had a partial response).[2-4] Responses to Proleukin may be evident as soon as 4 weeks after treatment.[2,5,*]

*Treatment with Proleukin is typically based on two 5-day cycles that constitute 1 course of therapy, with 9 days of rest in between. Patients who respond to Proleukin can go on to receive additional courses, while nonresponders are typically eligible for other treatment options.[2]

For injection
PROLEUKIN®
(aldesleukin) Recombinant IL-2

Learn more at
www.proleukin.com

References: 1. Clark JI, Curti B, Davis EJ, et al. Long-term progression-free survival of patients with metastatic melanoma or renal cell carcinoma following high-dose interleukin-2. *J Investig Med.* Published online Feb 4, 2021. doi: 10.1136/jim-2020-001650. **2.** Proleukin [package insert]. Yardley, PA: Clinigen, Inc; 2019. **3.** Fisher RI, Rosenberg SA, Fyfe G. Long-term survival update for high-dose recombinant interleukin-2 in patients with renal cell carcinoma. *Cancer J Sci Am.* 2000;6(suppl 1):S55-S57. **4.** Atkins MB, Kunkel L, Sznol M, Rosenberg SA. High-dose recombinant interleukin-2 therapy in patients with metastatic melanoma: long-term survival update. *Cancer J Sci Am.* 2000;6(suppl 1):S11-S14. **5.** Lindsey KR, Rosenberg SA, Sherry RM. Impact of the number of treatment courses on the clinical response of patients who receive high-dose bolus interleukin-2. *J Clin Oncol.* 2000;18(9):1954-1959.

CLINIGEN

PROLEUKIN® (aldesleukin)

for injection, for intravenous infusion

Rx Only

WARNINGS

Therapy with Proleukin® (aldesleukin) should be restricted to patients with normal cardiac and pulmonary functions as defined by thallium stress testing and formal pulmonary function testing. Extreme caution should be used in patients with a normal thallium stress test and a normal pulmonary function test who have a history of cardiac or pulmonary disease.

Proleukin should be administered in a hospital setting under the supervision of a qualified physician experienced in the use of anticancer agents. An intensive care facility and specialists skilled in cardiopulmonary or intensive care medicine must be available.

Proleukin administration has been associated with capillary leak syndrome (CLS) which is characterized by a loss of vascular tone and extravasation of plasma proteins and fluid into the extravascular space. CLS results in hypotension and reduced organ perfusion which may be severe and can result in death. CLS may be associated with cardiac arrhythmias (supraventricular and ventricular), angina, myocardial infarction, respiratory insufficiency requiring intubation, gastrointestinal bleeding or infarction, renal insufficiency, edema, and mental status changes.

Proleukin treatment is associated with impaired neutrophil function (reduced chemotaxis) and with an increased risk of disseminated infection, including sepsis and bacterial endocarditis. Consequently, preexisting bacterial infections should be adequately treated prior to initiation of Proleukin therapy. Patients with indwelling central lines are particularly at risk for infection with gram positive microorganisms. Antibiotic prophylaxis with oxacillin, nafcillin, ciprofloxacin, or vancomycin has been associated with a reduced incidence of staphylococcal infections.

Proleukin administration should be withheld in patients developing moderate to severe lethargy or somnolence; continued administration may result in coma.

DESCRIPTION

Aldesleukin, a human recombinant interleukin-2 product, is a highly purified protein with a molecular weight of approximately 15,300 daltons. The chemical name is des-alanyl-1, serine-125 human interleukin-2. Aldesleukin, a lymphokine, is produced by recombinant DNA technology using a genetically engineered *E. coli* strain containing an analog of the human interleukin-2 gene. Genetic engineering techniques were used to modify the human IL-2 gene, and the resulting expression clone encodes a modified human interleukin-2. This recombinant form differs from native interleukin-2 in the following ways: a) aldesleukin is not glycosylated because it is derived from *E. coli*; b) the molecule has no N-terminal alanine; the codon for this amino acid was deleted during the genetic engineering procedure; c) the molecule has serine substituted for cysteine at amino acid position 125; this was accomplished by site specific manipulation during the genetic engineering procedure; and d) the aggregation state of aldesleukin is likely to be different from that of native interleukin-2. The manufacturing process for aldesleukin involves fermentation in a defined medium containing tetracycline hydrochloride. The presence of the antibiotic is not detectable in the final product.

The *in vitro* biological activities of the native nonrecombinant molecule have been reproduced with aldesleukin.[1,2]

Proleukin (aldesleukin) for injection is a sterile, preservative-free white to off-white, lyophilized powder, which has a cake-like appearance, supplied in single-dose vials for intravenous administration after reconstitution. When reconstituted with 1.2 mL Sterile Water for Injection, USP, each mL contains 18 million International Units (1.1 mg) aldesleukin, mannitol (50 mg), sodium dodecyl sulfate (0.19 mg), buffered with disodium hydrogen phosphate dihydrate (1.12 mg) and sodium dihydrogen phosphate dihydrate (0.19 mg) to a pH of 7.5 (range 7.2 to 7.8).

Proleukin biological potency is determined by a lymphocyte proliferation bioassay and is expressed in International Units as established by the World Health Organization 1st International Standard for Interleukin-2 (human). The relationship between potency and protein mass is as follows:

18 million International Units Proleukin = 1.1 mg protein

CLINICAL PHARMACOLOGY

Proleukin® (aldesleukin) has been shown to possess the biological activities of human native interleukin-2.[1,2] *In vitro* studies performed on human cell lines demonstrate the immunoregulatory properties of Proleukin, including: a) enhancement of lymphocyte mitogenesis and stimulation of long-term growth of human interleukin-2 dependent cell lines; b) enhancement of lymphocyte cytotoxicity; c) induction of killer cell (lymphokine-activated (LAK) and natural (NK)) activity; and d) induction of interferon-gamma production.

The *in vivo* administration of Proleukin in animals and humans produces multiple immunological effects in a dose dependent manner. These effects include activation of cellular immunity with profound lymphocytosis, eosinophilia, and thrombocytopenia, and the production of cytokines including tumor necrosis factor, IL-1 and gamma interferon.[3] *In vivo* experiments in murine tumor models have shown inhibition of tumor growth.[4] The exact mechanism by which Proleukin mediates its antitumor activity in animals and humans is unknown.

Pharmacokinetics

Proleukin exists as biologically active, non-covalently bound microaggregates with an average size of 27 recombinant interleukin-2 molecules. The solubilizing agent, sodium dodecyl sulfate, may have an effect on the kinetic properties of this product.

The pharmacokinetic profile of Proleukin is characterized by high plasma concentrations following a short intravenous infusion, rapid distribution into the extravascular space and elimination from the body by metabolism in the kidneys with little or no bioactive protein excreted in the urine. Studies of intravenous Proleukin in sheep and humans indicate that upon completion of infusion, approximately 30% of the administered dose is detectable in plasma. This finding is consistent with studies in rats using radiolabeled Proleukin, which demonstrate a rapid (<1 min) uptake of the majority of the label into the lungs, liver, kidney, and spleen.

The serum half-life (T 1/2) curves of Proleukin remaining in the plasma are derived from studies done in 52 cancer patients following a 5-minute intravenous infusion. These patients were shown to have a distribution and elimination T 1/2 of 13 and 85 minutes, respectively.

Following the initial rapid organ distribution, the primary route of clearance of circulating Proleukin is the kidney. In humans and animals, Proleukin is cleared from the circulation by both glomerular filtration and peritubular extraction in the kidney.[5-8] This dual mechanism for delivery of Proleukin to the proximal tubule may account for the preservation of clearance in patients with rising serum creatinine values. Greater than 80% of the amount of Proleukin distributed to plasma, cleared from the circulation and presented to the kidney is metabolized to amino acids in the cells lining the proximal convoluted tubules. In humans, the mean clearance rate in cancer patients is 268 mL/min.

The relatively rapid clearance of Proleukin has led to dosage schedules characterized by frequent, short infusions. Observed serum levels are proportional to the dose of Proleukin.

CLINICAL STUDIES

Safety and efficacy were studied in a series of single and multicenter, historically controlled studies enrolling a total of 525 patients with metastatic renal cell carcinoma or melanoma. Eligible patients had an Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) of 0 or 1 and normal organ function as determined by cardiac stress test, pulmonary function tests, and creatinine ≤1.5 mg/dL. Studies excluded patients with brain metastases, active infections, organ allografts and diseases requiring steroid treatment.

The same treatment dose and schedule was employed in all studies demonstrating efficacy. Proleukin was given by 15 min intravenous infusion every 8 hours for up to 5 days (maximum of 14 doses). No treatment was given on days 6 to 14 and then dosing was repeated for up to 5 days on days 15 to 19 (maximum of 14 doses). These 2 cycles constituted 1 course of therapy. Patients could receive a maximum of 28 doses during a course of therapy. In practice >90% of patients had doses withheld. Doses were withheld for specific toxicities (See "**DOSAGE AND ADMINISTRATION**" section, "**Dose Modifications**" subsection and "**ADVERSE REACTIONS**" section).

Metastatic Renal Cell Cancer

Two hundred fifty-five patients with metastatic renal cell cancer (metastatic RCC) were treated with single agent Proleukin in 7 clinical studies conducted at 21 institutions. Metastatic RCC patients received a median of 20 of 28 scheduled doses of Proleukin.

In the renal cell cancer studies (n=255), objective response was seen in 37 (15%) patients, with 17 (7%) complete and 20 (8%) partial responders (See Table 1). The 95% confidence interval for objective response was 11% to 20%. Onset of tumor regression was observed as early as 4 weeks after completion of the first course of treatment, and in some cases, tumor regression continued for up to 12 months after the start of treatment. Responses were observed in both lung and non-lung sites (e.g., liver, lymph node, renal bed occurrences, soft tissue). Responses were also observed in patients with individual bulky lesions and high tumor burden.

TABLE 1: Proleukin Clinical Response Data

	Number of Responding Patients (response rate)	Median Response Duration in Months (range)
Metastatic RCC		
CR's	17 (7%)	80+* (7 to 131+)
PR's	20 (8%)	20 (3 to 126+)
PR's + CR's	37 (15%)	54 (3 to 131+)

(+) sign means ongoing

* Median duration not yet observed; a conservative value is presented which represents the minimum median duration of response.

Lack of efficacy with low dose Proleukin regimens

Sixty-five patients with metastatic renal cell cancer were enrolled in a single center, open label, non-randomized trial that sequentially evaluated the safety and anti-tumor activity of two low dose Proleukin regimens. The regimens administered 18 million International Units Proleukin as a single subcutaneous injection, daily for 5 days during week 1; Proleukin was then administered at 9 $x10^6$ International Units days 1-2 and 18 $x10^6$ International Units days 3-5, weekly for an additional 3 weeks (n=40) followed by a 2 week rest or 5 weeks (n=25) followed by a 3 week rest, for a maximum of 3 or 2 treatment cycles, respectively.

These low dose regimens yielded substantially lower and less durable responses than those observed with the approved regimen. Based on the level of activity, these low dose regimens are not effective.

Metastatic Melanoma

Two hundred seventy patients with metastatic melanoma were treated with single agent Proleukin in 8 clinical studies conducted at 22 institutions. Metastatic melanoma patients received a median of 18 of 28 scheduled doses of Proleukin during the first course of therapy. In the metastatic melanoma studies (n=270), objective response was seen in 43 (16%) patients, with 17 (6%) complete and 26 (10%) partial responders (See Table 2). The 95% confidence interval for objective response was 12% to 21%. Responses in metastatic melanoma patients were observed in both visceral and non-visceral sites (e.g., lung, liver, lymph node, soft tissue, adrenal, subcutaneous). Responses were also observed in patients with individual bulky lesions and large cumulative tumor burden.

TABLE 2: Proleukin CLINICAL RESPONSE DATA

	Number of Responding Patients (response rate)	Median Response Duration in Months (range)
Metastatic Melanoma		
CR's	17 (6%)	59+* (3 to 122+)
PR's	26 (10%)	6 (1 to 111+)
PR's + CR's	43 (16%)	9 (1 to 122+)

(+) sign means ongoing

* Median duration not yet observed; a conservative value is presented which represents the minimum median duration of response.

INDICATIONS AND USAGE

Proleukin® (aldesleukin) is indicated for the treatment of adults with metastatic renal cell carcinoma (metastatic RCC).

Proleukin is indicated for the treatment of adults with metastatic melanoma.

Careful patient selection is mandatory prior to the administration of Proleukin. See "**CONTRAINDICATIONS**", "**WARNINGS**" and "**PRECAUTIONS**" sections regarding patient screening, including recommended cardiac and pulmonary function tests and laboratory tests.

Evaluation of clinical studies to date reveals that patients with more favorable ECOG performance status (ECOG PS 0) at treatment initiation respond better to Proleukin, with a higher response rate and lower toxicity (See "**CLINICAL PHARMACOLOGY**" section, "**CLINICAL STUDIES**" section and "**ADVERSE REACTIONS**" section). Therefore, selection of patients for treatment should include assessment of performance status.

Experience in patients with ECOG PS >1 is extremely limited.

CONTRAINDICATIONS

Proleukin® (aldesleukin) is contraindicated in patients with a known history of hypersensitivity to interleukin-2 or any component of the Proleukin formulation.

Proleukin is contraindicated in patients with an abnormal thallium stress test or abnormal pulmonary function tests and those with organ allografts. Retreatment with Proleukin is contraindicated in patients who have experienced the following drug-related toxicities while receiving an earlier course of therapy:

- Sustained ventricular tachycardia (≥5 beats)
- Cardiac arrhythmias not controlled or unresponsive to management
- Chest pain with ECG changes, consistent with angina or myocardial infarction
- Cardiac tamponade
- Intubation for >72 hours
- Renal failure requiring dialysis >72 hours
- Coma or toxic psychosis lasting >48 hours
- Repetitive or difficult to control seizures
- Bowel ischemia/perforation
- GI bleeding requiring surgery

WARNINGS

See boxed "**WARNINGS**"

Because of the severe adverse events which generally accompany Proleukin® (aldesleukin) therapy at the recommended dosages, thorough clinical evaluation should be performed to identify patients with significant cardiac, pulmonary, renal, hepatic, or CNS impairment in whom Proleukin is contraindicated. Patients with normal cardiovascular, pulmonary, hepatic, and CNS function may experience serious, life threatening or fatal adverse events. Adverse events are frequent, often serious, and sometimes fatal.

Should adverse events, which require dose modification occur, dosage should be withheld rather than reduced (See "**DOSAGE AND ADMINISTRATION**" section, "**Dose Modifications**" subsection).

Proleukin has been associated with exacerbation of pre-existing or initial presentation of autoimmune disease and inflammatory disorders. Exacerbation of Crohn's disease, scleroderma, thyroiditis, inflammatory arthritis, diabetes mellitus, oculo-bulbar myasthenia gravis, crescentic IgA glomerulonephritis, cholecystitis, cerebral vasculitis, Stevens-Johnson syndrome and bullous pemphigoid, has been reported following treatment with IL-2.

All patients should have thorough evaluation and treatment of CNS metastases and have a negative scan prior to receiving Proleukin therapy. New neurologic signs, symptoms, and anatomic lesions following Proleukin therapy have been reported in patients without evidence of CNS metastases. Clinical manifestations included changes in mental status, speech difficulties, cortical blindness, limb or gait ataxia, hallucinations, agitation, obtundation, and coma. Radiological findings included multiple and, less commonly, single cortical lesions on MRI and evidence of demyelination. Neurologic signs and symptoms associated with Proleukin therapy usually improve after discontinuation of Proleukin therapy; however, there are reports of permanent neurologic defects. One case of possible cerebral vasculitis, responsive to dexamethasone, has been reported. In patients with known seizure disorders, extreme caution should be exercised as Proleukin may cause seizures.

PRECAUTIONS

General

Patients should have normal cardiac, pulmonary, hepatic, and CNS function at the start of therapy. (See "**PRECAUTIONS**" section, "**Laboratory Tests**" subsection). Capillary leak syndrome (CLS) begins immediately after Proleukin® (aldesleukin) treatment starts and is marked by increased capillary permeability to protein and fluids and reduced vascular tone. In most patients, this results in a concomitant drop in mean arterial blood pressure within 2 to 12 hours after the start of treatment. With continued therapy, clinically significant hypotension (defined as systolic blood pressure below 90 mm Hg or a 20 mm Hg drop from baseline systolic pressure) and hypoperfusion will occur. In addition, extravasation of protein and fluids into the extravascular space will lead to the formation of edema and creation of new effusions.

Medical management of CLS begins with careful monitoring of the patient's fluid and organ perfusion status. This is achieved by frequent determination of blood pressure and pulse, and by monitoring organ function, which includes assessment of mental status and urine output. Hypovolemia is assessed by catheterization and central pressure monitoring.

Flexibility in fluid and pressor management is essential for maintaining organ perfusion and blood pressure. Consequently, extreme caution should be used in treating patients with fixed requirements for large volumes of fluid (e.g., patients with hypercalcemia). Administration of IV fluids, either colloids or crystalloids is recommended for treatment of hypovolemia. Correction of hypovolemia may require large volumes of IV fluids but caution is required because unrestrained fluid administration may exacerbate problems associated with edema formation or effusions.

With extravascular fluid accumulation, edema is common and ascites, pleural or pericardial effusions may develop. Management of these events depends on a careful balancing of the effects of fluid shifts so that neither the consequences of hypovolemia (e.g., impaired organ perfusion) nor the consequences of fluid accumulations (e.g., pulmonary edema) exceed the patient's tolerance.

Clinical experience has shown that early administration of dopamine (1 to 5 mcg/kg/min) to patients manifesting capillary leak syndrome, before the onset of hypotension, can help to maintain organ perfusion particularly to the kidney and thus preserve urine output. Weight and urine output should be carefully monitored. If organ perfusion and blood pressure are not sustained by dopamine therapy, clinical investigators have increased the dose of dopamine to 6 to 10 mcg/kg/min or have added phenylephrine hydrochloride (1 to 5 mcg/kg/min) to low dose dopamine (See "**ADVERSE REACTIONS**" section). Prolonged use of pressors, either in combination or as individual agents, at relatively high doses, may be associated with cardiac rhythm disturbances. If there has been excessive weight gain or edema formation, particularly if associated with shortness of breath from pulmonary congestion, use of diuretics, once blood pressure has normalized, has been shown to hasten recovery. **NOTE: Prior to the use of any product mentioned, the physician should refer to the package insert for the respective product.**

Proleukin® (aldesleukin) treatment should be withheld for failure to maintain organ perfusion as demonstrated by altered mental status, reduced urine output, a fall in the systolic blood pressure below 90 mm Hg or onset of cardiac arrhythmias (See "**DOSAGE AND ADMINISTRATION**" section, "**Dose Modifications**" subsection). Recovery from CLS begins soon after cessation of Proleukin therapy. Usually, within a few hours, the blood pressure rises, organ perfusion is restored and reabsorption of extravasated fluid and protein begins.

Kidney and liver function are impaired during Proleukin treatment. Use of concomitant nephrotoxic or hepatotoxic medications may further increase toxicity to the kidney or liver.

Mental status changes including irritability, confusion, or depression which occur while receiving Proleukin may be indicators of bacteremia or early bacterial sepsis, hypoperfusion, occult CNS malignancy, or direct Proleukin-induced CNS toxicity. Alterations in mental status due solely to Proleukin therapy may progress for several days before recovery begins. Rarely, patients have sustained permanent neurologic deficits (See "**PRECAUTIONS**" section "**Drug Interactions**" subsection).

Exacerbation of pre-existing autoimmune disease or initial presentation of autoimmune and inflammatory disorders has been reported following Proleukin alone or in combination with interferon (See "**PRECAUTIONS**" section "**Drug Interactions**" subsection and "**ADVERSE REACTIONS**" section). Hypothyroidism, sometimes preceded by hyperthyroidism, has been reported following Proleukin treatment. Some of these patients required thyroid replacement therapy. Changes in thyroid function may be a manifestation of autoimmunity. Onset of symptomatic hyperglycemia and/or diabetes mellitus has been reported during Proleukin therapy.

Proleukin enhancement of cellular immune function may increase the risk of allograft rejection in transplant patients.

Serious Manifestations of Eosinophilia

Serious manifestations of eosinophilia involving eosinophilic infiltration of cardiac and pulmonary tissues can occur following Proleukin.

Laboratory Tests

The following clinical evaluations are recommended for all patients, prior to beginning treatment and then daily during drug administration.

- Standard hematologic tests-including CBC, differential and platelet counts
- Blood chemistries-including electrolytes, renal and hepatic function tests
- Chest x-rays

Serum creatinine should be ≤1.5 mg/dL prior to initiation of Proleukin treatment.

All patients should have baseline pulmonary function tests with arterial blood gases. Adequate pulmonary function should be documented (FEV_1 >2 liters or ≥75% of predicted for height and age) prior to initiating therapy.

All patients should be screened with a stress thallium study. Normal ejection fraction and unimpaired wall motion should be documented. If a thallium stress test suggests minor wall motion abnormalities further testing is suggested to exclude significant coronary artery disease.

Daily monitoring during therapy with Proleukin should include vital signs (temperature, pulse, blood pressure, and respiration rate), weight, and fluid intake and output. In a patient with a decreased systolic blood pressure, especially less than 90 mm Hg, constant cardiac rhythm monitoring should be conducted. If an abnormal complex or rhythm is seen, an ECG should be performed. Vital signs in these hypotensive patients should be taken hourly.

During treatment, pulmonary function should be monitored on a regular basis by clinical examination, assessment of vital signs and pulse oximetry. Patients with dyspnea or clinical signs of respiratory impairment (tachypnea or rales) should be further assessed with arterial blood gas determination. These tests are to be repeated as often as clinically indicated.

Cardiac function should be assessed daily by clinical examination and assessment of vital signs. Patients with signs or symptoms of chest pain, murmurs, gallops, irregular rhythm or palpitations should be further assessed with an ECG examination and cardiac enzyme evaluation. Evidence of myocardial injury, including findings compatible with myocardial infarction or myocarditis, has been reported. Ventricular hypokinesia due to myocarditis may be persistent for several months. If there is evidence of cardiac ischemia or congestive heart failure, Proleukin therapy should be held, and a repeat thallium study should be done.

Drug Interactions

Proleukin may affect central nervous function. Therefore, interactions could occur following concomitant administration of psychotropic drugs (e.g., narcotics, analgesics, antiemetics, sedatives, tranquilizers).

Concurrent administration of drugs possessing nephrotoxic (e.g., aminoglycosides, indomethacin), myelotoxic (e.g., cytotoxic chemotherapy), cardiotoxic (e.g., doxorubicin) or hepatotoxic (e.g., methotrexate, asparaginase) effects with Proleukin may increase toxicity in these organ systems. The safety and efficacy of Proleukin in combination with any antineoplastic agents have not been established.

In addition, reduced kidney and liver function secondary to Proleukin treatment may delay elimination of concomitant medications and increase the risk of adverse events from those drugs.

Hypersensitivity reactions have been reported in patients receiving combination regimens containing sequential high dose Proleukin and antineoplastic agents, specifically, dacarbazine, cis-platinum, tamoxifen and interferon-alfa. These reactions consisted of erythema, pruritus, and hypotension and occurred within hours of administration of chemotherapy. These events required medical intervention in some patients.

Myocardial injury, including myocardial infarction, myocarditis, ventricular hypokinesia, and severe rhabdomyolysis appear to be increased in patients receiving Proleukin and interferon-alfa concurrently.

Exacerbation or the initial presentation of a number of autoimmune and inflammatory disorders has been observed following concurrent use of interferon-alfa and Proleukin, including crescentic IgA glomerulonephritis, oculo-bulbar myasthenia gravis, inflammatory arthritis, thyroiditis, bullous pemphigoid, and Stevens-Johnson syndrome.

Although glucocorticoids have been shown to reduce Proleukin-induced side effects including fever, renal insufficiency, hyperbilirubinemia, confusion, and dyspnea, concomitant administration of these agents with Proleukin may reduce the antitumor effectiveness of Proleukin and thus should be avoided.[12]

Beta-blockers and other antihypertensives may potentiate the hypotension seen with Proleukin.

Delayed Adverse Reactions to Iodinated Contrast Media

A review of the literature revealed that 12.6% (range 11-28%) of 501 patients treated with various interleukin-2 containing regimens who were subsequently administered radiographic iodinated contrast media experienced acute, atypical adverse reactions. The onset of symptoms usually occurred within hours (most commonly 1 to 4 hours) following the administration of contrast media. These reactions include fever, chills, nausea, vomiting, pruritus, rash, diarrhea, hypotension, edema, and oliguria. Some clinicians have noted that these reactions resemble the immediate side effects caused by interleukin-2 administration, however the cause of contrast reactions after interleukin-2 therapy is unknown. Most events were reported to occur when contrast media was given within 4 weeks after the last dose of interleukin-2. These events were also reported to occur when contrast media was given several months after interleukin-2 treatment.[13]

Carcinogenesis, Mutagenesis, Impairment of Fertility

There have been no studies conducted assessing the carcinogenic or mutagenic potential of Proleukin.

There have been no studies conducted assessing the effect of Proleukin on fertility. It is recommended that this drug not be administered to fertile persons of either gender not practicing effective contraception.

Pregnancy

Pregnancy Category C.

Proleukin has been shown to have embryolethal effects in rats when given in doses at 27 to 36 times the human dose (scaled by body weight). Significant maternal toxicities were observed in pregnant rats administered Proleukin by IV injection at doses 2.1 to 36 times higher than the human dose during critical period of organogenesis. No evidence of teratogenicity was observed other than that attributed to maternal toxicity. There are no adequate well-controlled studies of Proleukin in pregnant women. Proleukin should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from Proleukin, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

Pediatric Use

Safety and effectiveness in children under 18 years of age have not been established.

Geriatric Use

There were a small number of patients aged 65 and over in clinical trials of Proleukin; experience is limited to 27 patients, eight with metastatic melanoma and nineteen with metastatic renal cell carcinoma. The response rates were similar in patients 65 years and over as compared to those less than 65 years of age. The median number of courses and the median number of doses per course were similar between older and younger patients.

Proleukin is known to be substantially excreted by the kidney, and the risk of toxic reactions to this drug may be greater in patients with impaired renal function. The pattern of organ system toxicity and the proportion of patients with severe toxicities by organ system were generally similar in patients 65 and older and younger patients. There was a trend, however, towards an increased incidence of severe urogenital toxicities and dyspnea in the older patients.

ADVERSE REACTIONS

The rate of drug-related deaths in the 255 metastatic RCC patients who received single-agent Proleukin® (aldesleukin) was 4% (11/255); the rate of drug-related deaths in the 270 metastatic melanoma patients who received single-agent Proleukin was 2% (6/270).

The following data on common adverse events (reported in greater than 10% of patients, any grade), presented by body system, decreasing frequency and by preferred term (COSTART) are based on 525 patients (255 with renal cell cancer and 270 with metastatic melanoma) treated with the recommended infusion dosing regimen.

TABLE 3: ADVERSE EVENTS OCCURRING IN ≥10% OF PATIENTS (n=525)

Body System	% Patients	Body System	%Patients
Body as a Whole		**Metabolic and Nutritional Disorders**	
Chills	52	Bilirubinemia	40
Fever	29	Creatinine increase	33
Malaise	27	Peripheral edema	28
Asthenia	23	SGOT increase	23
Infection	13	Weight gain	16
Pain	12	Edema	15
Abdominal pain	11	Acidosis	12
Abdomen enlarged	10	Hypomagnesemia	12
Cardiovascular		Hypocalcemia	11
Hypotension	71	Alkaline phosphatase increase	10
Tachycardia	23	**Nervous**	
Vasodilation	13	Confusion	34
Supraventricular tachycardia	12	Somnolence	22
Cardiovascular disorder[a]	11	Anxiety	12
Arrhythmia	10	Dizziness	11
Digestive		**Respiratory**	
Diarrhea	67	Dyspnea	43
Vomiting	50	Lung disorder[b]	24
Nausea	35	Respiratory disorder[c]	11
Stomatitis	22	Cough increase	11
Anorexia	20	Rhinitis	10
Nausea and vomiting	19	**Skin and Appendages**	
Hemic and Lymphatic		Rash	42
Thrombocytopenia	37	Pruritus	24
Anemia	29	Exfoliative dermatitis	18
Leukopenia	16	**Urogenital**	
		Oliguria	63

[a] Cardiovascular disorder: fluctuations in blood pressure, asymptomatic ECG changes, CHF.
[b] Lung disorder: physical findings associated with pulmonary congestion, rales, rhonchi.
[c] Respiratory disorder: ARDS, CXR infiltrates, unspecified pulmonary changes.

The following data on life-threatening adverse events (reported in greater than 1% of patients, grade 4), presented by body system, and by preferred term (COSTART) are based on 525 patients (255 with renal cell cancer and 270 with metastatic melanoma) treated with the recommended infusion dosing regimen.

TABLE 4: LIFE-THREATENING (GRADE 4) ADVERSE EVENTS (n= 525)

Body System	# (%) Patients	Body System	# (%) Patients
Body as a Whole		**Metabolic and Nutritional Disorders**	
Fever	5 (1%)	Bilirubinemia	13 (2%)
Infection	7 (1%)	Creatinine increase	5 (1%)
Sepsis	6 (1%)	SGOT increase	3 (1%)
Cardiovascular		Acidosis	4 (1%)
Hypotension	15 (3%)	**Nervous**	
Supraventricular tachycardia	3 (1%)	Confusion	5 (1%)
Cardiovascular disorder[a]	7 (1%)	Stupor	3 (1%)
Myocardial infarct	7 (1%)	Coma	8 (2%)
Ventricular tachycardia	5 (1%)	Psychosis	7 (1%)
Cardiac arrest	4 (1%)	**Respiratory**	
Digestive		Dyspnea	5 (1%)
Diarrhea	10 (2%)	Respiratory disorder[c]	14 (3%)
Vomiting	7 (1%)	Apnea	5 (1%)
Hemic and Lymphatic		**Urogenital**	
Thrombocytopenia	5 (1%)	Oliguria	33 (6%)
Coagulation disorder[b]	4 (1%)	Anuria	25 (5%)
		Acute kidney failure	3 (1%)

[a] Cardiovascular disorder: fluctuations in blood pressure.
[b] Coagulation disorder: intravascular coagulopathy.
[c] Respiratory disorder: ARDS, respiratory failure, intubation.

The following life-threatening (grade 4) events were reported by <1% of the 525 patients: hypothermia; shock; bradycardia; ventricular extrasystoles; myocardial ischemia; syncope; hemorrhage; atrial arrhythmia; phlebitis; AV block second degree; endocarditis; pericardial effusion; peripheral gangrene; thrombosis; coronary artery disorder; stomatitis; nausea and vomiting; liver function tests abnormal; gastrointestinal hemorrhage; hematemesis; bloody diarrhea; gastrointestinal disorder; intestinal perforation; pancreatitis; anemia; leukopenia; leukocytosis; hypocalcemia; alkaline phosphatase increase; BUN increase; hyperuricemia; NPN increase; respiratory acidosis; somnolence; agitation; neuropathy; paranoid reaction; convulsion; grand mal convulsion; delirium; asthma, lung edema; hyperventilation; hypoxia; hemoptysis; hypoventilation; pneumothorax; mydriasis; pupillary disorder; kidney function abnormal; kidney failure; acute tubular necrosis.

In an additional population of greater than 1,800 patients treated with Proleukin-based regimens using a variety of doses and schedules (e.g., subcutaneous, continuous infusion, administration with LAK cells) the following serious adverse events were reported: duodenal ulceration; bowel necrosis; myocarditis; supraventricular tachycardia; permanent or transient blindness secondary to optic neuritis; transient ischemic attacks; meningitis; cerebral edema; pericarditis; allergic interstitial nephritis; tracheo-esophageal fistula.

In the same clinical population, the following fatal events each occurred with a frequency of <1%: malignant hyperthermia; cardiac arrest; myocardial infarction; pulmonary emboli; stroke; intestinal perforation; liver or renal failure; severe depression leading to suicide; pulmonary edema; respiratory arrest; respiratory failure. In patients with both metastatic RCC and metastatic melanoma, those with ECOG PS of 1 or higher had a higher treatment-related mortality and serious adverse events.

Most adverse reactions are self-limiting and, usually, but not invariably, reverse or improve within 2 or 3 days of discontinuation of therapy. Examples of adverse reactions with permanent sequelae include: myocardial infarction, bowel perforation/infarction, and gangrene.

Immunogenicity

Serum samples from patients in the clinical studies were tested by enzyme-linked immunosorbent assay (ELISA) for anti-aldesleukin antibodies. Low titers of anti-aldesleukin antibodies were detected in 57 of 77 (74%) patients with metastatic renal cell carcinoma treated with an every 8-hour PROLEUKIN regimen and in 33 of 50 (66%) patients with metastatic melanoma treated with a variety of intravenous regimens. In a separate study, the effect of immunogenicity on the pharmacokinetics of aldesleukin was evaluated in 13 patients. Following the first cycle of therapy, comparing the geometric mean aldesleukin exposure (AUC) Day 15 to Day 1, there was an average 68% increase in 11 patients who developed anti-aldesleukin antibodies and no change was observed in the antibody-negative patients (n=2). Overall, neutralizing antibodies were detected in 1 patient. The impact of antialdesleukin antibody formation on clinical efficacy and safety of Proleukin is unknown.

Immunogenicity assay results are highly dependent on several factors including assay sensitivity and specificity, assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of incidence of antibodies to PROLEUKIN with the incidence of antibodies to other products may be misleading.

Post Marketing Experience

The following adverse reactions have been identified during post-approval use of Proleukin. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

- Blood and lymphatic system: neutropenia, febrile neutropenia, eosinophilia, lymphocytopenia
- Cardiac: cardiomyopathy, cardiac tamponade
- Endocrine: hyperthyroidism
- Gastrointestinal: gastritis, intestinal obstruction, colitis
- General and administration site conditions: injection site necrosis
- Hepatobiliary: hepatitis, hepatosplenomegaly, cholecystitis
- Immune system: anaphylaxis, angioedema, urticaria
- Infections and infestations: pneumonia (bacterial, fungal, viral), fatal endocarditis, cellulitis
- Musculoskeletal and connective tissue: myopathy, myositis, rhabdomyolysis
- Nervous system: cerebral lesions, encephalopathy, extrapyramidal syndrome, neuralgia, neuritis, demyelinating neuropathy
- Psychiatric: insomnia
- Vascular: hypertension, fatal subdural and subarachnoid hemorrhage, cerebral hemorrhage, retroperitoneal hemorrhage

Exacerbation or initial presentation of a number of autoimmune and inflammatory disorders have been reported (See "**WARNINGS**" section, "**PRECAUTIONS**" section, "**Drug Interactions**" subsection). Persistent but nonprogressive vitiligo has been observed in malignant melanoma patients treated with interleukin-2. Synergistic, additive and novel toxicities have been reported with Proleukin used in combination with other drugs. Novel toxicities include delayed adverse reactions to iodinated contrast media and hypersensitivity reactions to antineoplastic agents (See "**PRECAUTIONS**" section, "**Drug Interactions**" subsection).

Experience has shown the following concomitant medications to be useful in the management of patients on Proleukin therapy: a) standard antipyretic therapy, including nonsteroidal anti-inflammatories (NSAIDs), started immediately prior to Proleukin to reduce fever. Renal function should be monitored as some NSAIDs may cause synergistic nephrotoxicity; b) meperidine used to control the rigors associated with fever; c) H_2 antagonists given for prophylaxis of gastrointestinal irritation and bleeding; d) antiemetics and antidiarrheals used as needed to treat other gastrointestinal side effects. Generally these medications were discontinued 12 hours after the last dose of Proleukin.

Patients with indwelling central lines have a higher risk of infection with gram positive organisms.[9-11] A reduced incidence of staphylococcal infections in Proleukin studies has been associated with the use of antibiotic prophylaxis which includes the use of oxacillin, nafcillin, ciprofloxacin, or vancomycin. Hydroxyzine or diphenhydramine has been used to control symptoms from pruritic rashes and continued until resolution of pruritus. Topical creams and ointments should be applied as needed for skin manifestations. Preparations containing a steroid (e.g., hydrocortisone) should be avoided. **NOTE: Prior to the use of any product mentioned, the physician should refer to the package insert for the respective product.**

OVERDOSAGE

Side effects following the use of Proleukin® (aldesleukin) appear to be dose-related. Exceeding the recommended dose has been associated with a more rapid onset of expected dose-limiting toxicities. Symptoms which persist after cessation of Proleukin should be monitored and treated supportively. Life-threatening toxicities may be ameliorated by the intravenous administration of dexamethasone, which may also result in loss of the therapeutic effects of Proleukin.[12] **NOTE: Prior to the use of dexamethasone, the physician should refer to the package insert for this product.**

DOSAGE AND ADMINISTRATION

The recommended Proleukin® (aldesleukin) treatment regimen is administered by a 15-minute intravenous infusion every 8 hours. Before initiating treatment, carefully review the "**INDICATIONS AND USAGE**", "**CONTRAINDICATIONS**", "**WARNINGS**", "**PRECAUTIONS**", and "**ADVERSE REACTIONS**" sections, particularly regarding patient selection, possible serious adverse events, patient monitoring and withholding dosage. The following schedule has been used to treat adult patients with metastatic renal cell carcinoma (metastatic RCC) or metastatic melanoma. Each course of treatment consists of two 5-day treatment cycles separated by a rest period.

600,000 International Units/kg (0.037 mg/kg) dose administered every 8 hours by a 15-minute intravenous infusion for a maximum of 14 doses. Following 9 days of rest, the schedule is repeated for another 14 doses, for a maximum of 28 doses per course, as tolerated. During clinical trials, doses were frequently withheld for toxicity (See "**CLINICAL STUDIES**" section and "**Dose Modifications**" subsection). Metastatic RCC patients treated with this schedule received a median of 20 of the 28 doses during the first course of therapy. Metastatic melanoma patients received a median of 18 doses during the first course of therapy.

Retreatment

Patients should be evaluated for response approximately 4 weeks after completion of a course of therapy and again immediately prior to the scheduled start of the next treatment course. Additional courses of treatment should be given to patients only if there is some tumor shrinkage following the last course and retreatment is not contraindicated (See "**CONTRAINDICATIONS**" section). Each treatment course should be separated by a rest period of at least 7 weeks from the date of hospital discharge.

Dose Modifications

Dose modification for toxicity should be accomplished by withholding or interrupting a dose rather than reducing the dose to be given. Decisions to stop, hold, or restart Proleukin therapy must be made after a global assessment of the patient. With this in mind, the following guidelines should be used:

Retreatment with Proleukin is contraindicated in patients who have experienced the following toxicities:

Body System	
Cardiovascular	Sustained ventricular tachycardia (≥5 beats)
	Cardiac rhythm disturbances not controlled or unresponsive to management
	Chest pain with ECG changes, consistent with angina or myocardial infarction
	Cardiac tamponade
Respiratory	Intubation for >72 hours
Urogenital	Renal failure requiring dialysis >72 hours
Nervous	Coma or toxic psychosis lasting >48 hours
	Repetitive or difficult to control seizures
Digestive	Bowel ischemia/perforation
	GI bleeding requiring surgery

Doses should be held and restarted according to the following:

Body System	Hold dose for	Subsequent doses may be given if
Cardiovascular	Atrial fibrillation, supraventricular tachycardia or bradycardia that requires treatment or is recurrent or persistent	Patient is asymptomatic with full recovery to normal sinus rhythm
	Systolic bp <90 mm Hg with increasing requirements for pressors	Systolic bp ≥90 mm Hg and stable or improving requirements for pressors
	Any ECG change consistent with MI, ischemia or myocarditis with or without chest pain; suspicion of cardiac ischemia	Patient is asymptomatic, MI and myocarditis have been ruled out, clinical suspicion of angina is low; there is no evidence of ventricular hypokinesia
Respiratory	O_2 saturation <90%	O_2 saturation >90%
Nervous	Mental status changes, including moderate confusion or agitation	Mental status changes completely resolved
Body as a Whole	Sepsis syndrome, patient is clinically unstable	Sepsis syndrome has resolved, patient is clinically stable, infection is under treatment
Urogenital	Serum creatinine >4.5 mg/dL or a serum creatinine of ≥4 mg/dL in the presence of severe volume overload, acidosis, or hyperkalemia	Serum creatinine <4 mg/dL and fluid and electrolyte status is stable
	Persistent oliguria, urine output of <10 mL/hour for 16 to 24 hours with rising serum creatinine	Urine output >10 mL/hour with a decrease of serum creatinine >1.5 mg/dL or normalization of serum creatinine
Digestive	Signs of hepatic failure including encephalopathy, increasing ascites, liver pain, hypoglycemia	All signs of hepatic failure have resolved*
	Stool guaiac repeatedly >3-4+	Stool guaiac negative
Skin	Bullous dermatitis or marked worsening of pre-existing skin condition, avoid topical steroid therapy	Resolution of all signs of bullous dermatitis

* Discontinue all further treatment for that course. A new course of treatment, if warranted, should be initiated no sooner than 7 weeks after cessation of adverse event and hospital discharge.

Reconstitution and Dilution Directions: Reconstitution and dilution procedures other than those recommended may alter the delivery and/or pharmacology of Proleukin and thus should be avoided.

1. Proleukin® (aldesleukin) is a sterile, white to off-white, preservative-free, lyophilized powder suitable for IV infusion upon reconstitution and dilution. **EACH VIAL CONTAINS 22 MILLION International Units (1.3 mg) OF PROLEUKIN AND SHOULD BE RECONSTITUTED ASEPTICALLY WITH 1.2 mL OF STERILE WATER FOR INJECTION, USP. WHEN RECONSTITUTED AS DIRECTED, EACH mL CONTAINS 18 MILLION International Units (1.1 mg) OF PROLEUKIN.** The resulting solution should be a clear, colorless to slightly yellow liquid. The vial is for single-use only and any unused portion should be discarded.
2. During reconstitution, the Sterile Water for Injection, USP should be directed at the side of the vial and the contents gently swirled to avoid excess foaming. DO NOT SHAKE.
3. The dose of Proleukin, reconstituted with Sterile Water for Injection, USP (without preservative) should be diluted aseptically in 50 mL of 5% Dextrose Injection, USP (D5W) and infused over a 15-minute period.
   In cases where the total dose of Proleukin is 1.5 mg or less (e.g., a patient with a body weight of less than 40 kilograms), the dose of Proleukin should be diluted in a smaller volume of D5W. Concentrations of Proleukin below 0.03 mg/mL and above 0.07 mg/mL have shown increased variability in drug delivery. Dilution and delivery of Proleukin outside of this concentration range should be avoided.
4. Glass bottles and plastic (polyvinyl chloride) bags have been used in clinical trials with comparable results. It is recommended that plastic bags be used as the dilution container since experimental studies suggest that use of plastic containers results in more consistent drug delivery. **In-line filters should not be used when administering Proleukin.**
5. Before and after reconstitution and dilution, store in a refrigerator at 2° to 8°C (36° to 46°F). Do not freeze. Administer Proleukin within 48 hours of reconstitution. The solution should be brought to room temperature prior to infusion in the patient.
6. Reconstitution or dilution with Bacteriostatic Water for Injection, USP, or 0.9% Sodium Chloride Injection, USP should be avoided because of increased aggregation. Proleukin should not be coadministered with other drugs in the same container.
7. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

HOW SUPPLIED

Proleukin® (aldesleukin) is supplied in individually boxed single-dose vials. Each vial contains 22 million International Units of Proleukin. Discard unused portion.

NDC 76310-022-01 Individually boxed single-dose vial

Store vials of lyophilized Proleukin in a refrigerator at 2° to 8°C (36° to 46°F). PROTECT FROM LIGHT. Store in carton until time of use.

Reconstituted or diluted Proleukin is stable for up to 48 hours at refrigerated and room temperatures, 2° to 25°C (36° to 77°F). However, since this product contains no preservative, the reconstituted and diluted solutions should be stored in the refrigerator.

Do not use beyond the expiration date printed on the vial. **NOTE:** This product contains no preservative.

Rx Only

REFERENCES

1. Doyle MV, Lee MT, Fong S. Comparison of the biological activities of human recombinant interleukin-2^{125} and native interleukin-2. *J Biol Response Mod* 1985; **4**:96-109. **2.** Ralph P, Nakoinz I, Doyle M, et al. Human B and T lymphocyte stimulating properties of interleukin-2 (IL-2) muteins. In: *Immune Regulation By Characterized Polypeptides.* Alan R. Liss, Inc. 1987; 453-62. **3.** Winkelhake JL and Gauny SS. Human recombinant interleukin-2 as an experimental therapeutic. *Pharmacol Rev* 1990; **42**:1-28. **4.** Rosenberg SA, Mule JJ, Spiess PJ, et al. Regression of established pulmonary metastases and subcutaneous tumor mediated by the systemic administration of high-dose recombinant interleukin-2. *J Exp Med* 1985; **161**:1169-88. **5.** Konrad MW, Hemstreet G, Hersh EM, et al. Pharmacokinetics of recombinant interleukin-2 in humans. *Cancer Res* 1990; **50**:2009-17. **6.** Donohue JH and Rosenberg SA. The fate of interleukin-2 after in vivo administration. *J Immunol* 1983; **130**:2203-8. **7.** Koths K, Halenbeck R. Pharmacokinetic studies on ^{35}S-labeled recombinant interleukin-2 in mice. In: Sorg C and Schimpl A, eds. *Cellular and Molecular Biology of Lymphokines.* Academic Press: Orlando, FL, 1985;779. **8.** Gibbons JA, Luo ZP, Hansen ER, et al. Quantitation of the renal clearance of interleukin-2 using nephrectomized and ureter ligated rats. *J Pharmacol Exp Ther* 1995; **272**: 119-125. **9.** Bock SN, Lee RE, Fisher B, et al. A prospective randomized trial evaluating prophylactic antibiotics to prevent triple-lumen catheter-related sepsis in patients treated with immunotherapy. *J Clin Oncol* 1990; 8:161-69. **10.** Hartman LC, Urba WJ, Steis RG, et al. Use of prophylactic antibiotics for prevention of intravascular catheter-related infections in interleukin-2-treated patients. *J Natl Cancer Inst* 1989; 81:1190-93. **11.** Snydman DR, Sullivan B, Gill M, et al. Nosocomial sepsis associated with interleukin-2. *Ann Intern Med* 1990; 112:102-07. **12.** Mier JW, Vachino G, Klempner MS, et al. Inhibition of interleukin-2-induced tumor necrosis factor release by dexamethasone: Prevention of an acquired neutrophil chemotaxis defect and differential suppression of interleukin-2 associated side effects. *Blood* 1990; 76:1933-40. **13.** Choyke PL, Miller DL, Lotze MT, et al. Delayed reactions to contrast media after interleukin-2 immunotherapy. *Radiology* 1992; 183:111-114.

Manufactured by: Clinigen, Inc. Yardley, PA 19067
U.S. License No. 2154
At
Boehringer Ingelheim Pharma Biberach/Riss, Germany

For additional information, contact Clinigen, Inc.
1-877-PROLEUKIN (1-877-776-5385)

REV: September 2019 US-PRO-2020-0008-01

ABOUT THE COVER

A graphical illustration depicting therapeutic perspectives of pooled DNA sequencing strategy in Renal Cell Carcinoma. This strategy significantly increases mutation detection while reducing clonality misattribution and overcomes intrinsic genetic heterogeneity. This leads to an increased fraction of patients identified with therapeutically actionable mutations and improved patient risk stratification.

KCJ CONTENTS

PUBLISHING STAFF
Senthil Samy, PhD., Executive Editor & Publisher
Vinu Jyothi, MD, MPH., Director, Clinical Strategy
Stu Chapman, Editorial Consultant
Susan Hirschhaut, Adv Initiatives Director
Matthew McConnell, Design Director

Editorial Office
517 Belle Gate Pl, Cary, NC 27519 USA
Email: office@kidney-cancer-journal.com

OPEN ACCESS JOURNAL

Kidney Cancer Journal (ISSN 1933-0863) is published quarterly by BMG (BioMedz Global). Kidney Cancer Magazine is a federally Registered Trademark of BMG.

A USA Based Publication

Resource-Efficient Pooled Sequencing Expands Translational Impact in Solid Tumors

Renzo G. DiNatale[a,b,c], Roy Mano[c], Vladimir Makarov[b], Nicole Rusk[a], Esther Drill[d], Andrew Winer[e], Alexander Sankin[f], Angela Yoo[c,e], Benjamin A. Freeman[c], James J. Hsieh[g], Ying-Bei Chen[h], Jonathan A. Coleman[c,] Michael Berger[i], Irina Ostrovnaya[d], Timothy A. Chan[b], Paul Russo[c], Ed Reznik * [a,i], A. Ari Hakimi * [b,c]

[a] *Computational Oncology Service, Epidemiology & Biostatistics Department, Memorial Sloan Kettering Cancer Center, New York, USA.*
[b] *Immunogenomics and Precision Oncology Platform, Memorial Sloan Kettering Cancer Center, New York, USA.*
[c] *Urology Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, USA.*
[d] *Biostatistics, Epidemiology & Biostatistics Department, Memorial Sloan Kettering Cancer Center, New York, USA.*
[e] *Department of Urology, SUNY Downstate Health Sciences University, Brooklyn, NY.*
[f] *Department of Urology, Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY.*
[g] *Department of Medicine, University of Washington, Washington D.C., USA.*
[h] *Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, USA.*
[i] *Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center, New York, USA.*
* These authors contributed equally.

https://doi.org/10.52733/KCJ18n2.a1

Intratumoral genetic heterogeneity (ITH) poses a significant challenge to utilizing sequencing for decision making in the management of cancer. Although sequencing of multiple tumor regions can address the pitfalls of ITH, it does so at a significant increase in cost and resource utilization. We propose a pooled multiregional sequencing strategy, whereby DNA aliquots from multiple tumor regions are mixed prior to sequencing, as a cost-effective strategy to boost translational value by addressing ITH while preserving valuable residual tissue for secondary analysis. Focusing on kidney cancer, we demonstrate that DNA pooling from as few as two regions significantly increases mutation detection while reducing clonality misattribution. This leads to an increased fraction of patients identified with therapeutically actionable mutations, improved patient risk stratification, and improved inference of evolutionary trajectories with an accuracy comparable to bona fide multiregional sequencing. The same approach applied to non-small-cell lung cancer data substantially improves tumor mutational burden (TMB) detection. Our findings demonstrate that pooled DNA sequencing strategies are a cost-effective alternative to address intrinsic genetic heterogeneity in clinical settings.

KEYWORDS: • cancer genomics • cancer evolution • intratumoral heterogeneity • next-generation sequencing • somatic mutation • clonality

Clear cell renal cell carcinoma (ccRCC), the most common and aggressive form of kidney cancer, is characterized by extensive intratumoral heterogeneity (ITH) whereby driver mutations frequently arise only in a subset of tumor cells[1–3]. As a result of ITH, clinically informative but subclonal mutations are commonly missed by the standard practice (at our institution[4] and others[5]) of sequencing single tumor regions. In a landmark multiregional sequencing study of 101 ccRCC tumors, the TRACERx consortium reported that fifty-six percent of all detected mutations were subclonal[6], and ~20% of subclonal mutations had demonstrable clinical value either for prognostication in clinical risk models (TP53, BAP1, and PBRM1)[7], or as criteria for administration of targeted therapy (MTOR, TSC1, and PTEN)[8] (Figure 1A). Single region sequencing places a hard constraint on the sensitivity to detect and study mutations for two reasons: somatic mutation dropout (i.e. absence of a mutation in the particular tumor region sampled) and erroneous clonality assertions (i.e. attributing mutations as clonal when in fact they are only subclonal or vice versa). Multiregional sequencing strategies address ITH by sequencing the genomic material of several spatially separated regions of the same tumor[9]. However, due to the added sequencing expenses, this approach becomes prohibitively costly as the number of regions increases, limiting its use in practice.

We reasoned that a more cost-effective approach to quantitatively managing ITH would be to pool samples from many regions together into a single "pseudo-bulk" before library construction (Figure 1B). Doing so would potentially ameliorate mutation dropout by increasing the likelihood of capturing a subclonal mutation while reducing the misattribution of clonal status to mutations present only in single regions of the tumor. The benefits of a pooled approach would come at several costs: first, from diluting the sequencing bandwidth devoted to individual region, and second, from loss of spatial information that would be obtained from bona fide multiregional sequencing. However, complete loss of spatial information could potentially be avoided (with an increase in cost and overhead) by barcoding DNA libraries before sequencing, an approach that has previously been demonstrated by several

Correspondence: *Ed Reznik: reznike@mskcc.org; A. Ari Hakim: hakimia@mskcc.org*

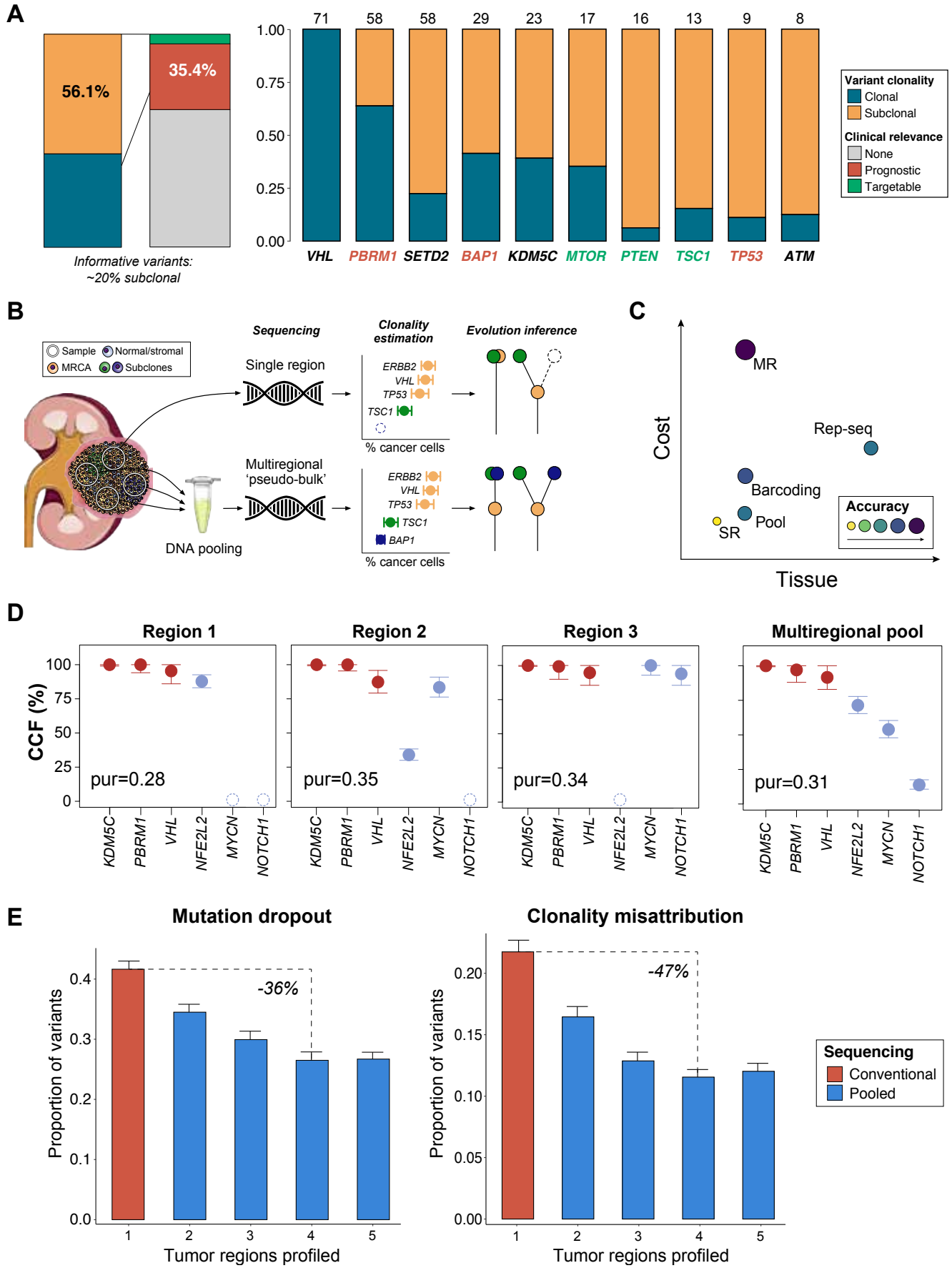

Figure 1 legend on next page

investigators[10]. Furthermore, pooling of tumor regions preserves precious tumor tissue which could be used for further molecular, immunohistochemical, or other profiling, and therefore is a material-efficient alternative to fully unbiased representative sequencing[11]. Direct pooling of DNA samples thus represents a flexible, cost-efficient middle-ground strategy that can be readily implemented into current pipelines without requiring additional expertise or reagents (Figure 1C).

We examined the feasibility of pooled sequencing using a deep, targeted clinical sequencing platform. For each of six ccRCC tumors, six spatially discrete regions were selected and pooled into a single sample. In parallel, we sequenced separate aliquots of the same tumor regions to standard depth, generating a ground-truth set of variant calls (Figure 1D and Supplementary figure 1a). One case (RCC006) had no variants identified in any region and was not included in the mutational analysis (Supplementary table 1, 2). Multiregional pooled sequencing of six regions at an average depth of ~900x (150x/region) resulted in a mutation dropout rate of 4.3% (1/23 variants) and a clonality error rate of 4.5% (1/22 variants) (Supplementary figure 2a). Compared to single region profiling, pooled multi-regional sequencing showed a 12% lower dropout rate (95% CI: 2.0 - 22.4%, Welch t-test, p=0.02) and a 13% lower clonality error rate (95% CI: 1.2 - 24.9%, Welch t-test, p=0.03) (Supplementary Figure 2b). Reduction in clonality misattribution was robust with the chosen cancer-cell fraction (CCF) threshold, with an estimated Matthew's correlation coefficient (MCC) of 0.73 (with +1 indicating perfect prediction and -1 complete disagreement) (Supplementary figure 2c, d). Notably, all the mutations missed/misclassified were present in the tumor with the highest regional variability in purity (RCC004, with a variance 5-fold higher than the average, $\sigma 2 = 0.05$ vs 0.01), and none of the pooled samples had purity estimates below our quality threshold (compared to 14%, or 5/36, of the regions profiled separately). These findings demonstrate an additional potential advantage of pooled sequencing, i.e. the possibility to reduce sample failure rates during clinical sequencing.

To validate our findings and further assess the utility of this sequencing strategy, we analyzed multiregional sequencing data from the TRACERx consortium. The validation cohorts consisted of 101 individuals with a ccRCC diagnosis (median, 8 tumor regions range, 2-75) profiled with a sequencing panel targeting 110 cancer genes at a median depth of 612x (range, 105–1,520x) (TRACERx RCC cohort,6), and 100 patients with non-small cell lung cancer (TRACERx NSCLC cohort), profiled with exome sequencing at a median depth of 431x (range 83-986x) for tumor regions and 415x (range 107-765x) for the matched germline (median, 3 tumor regions, range 2-8)[12] (Supplementary table 3). From our data, we confirmed that tumor purity estimates in DNA pools were predictable in silico to high accuracy using tumor purity from single regions (Supplementary figure 2e) Next, we simulated pooled sequencing in the TRACERx data (at equivalent depth to single-region sequencing) using a bootstrapping procedure (see Methods). Outcomes were then calculated on each random sample and averaged to produce region-number-specific estimates.

Pooled sequencing substantially decreased mutation dropout relative to single region profiling, even with the addition of just a single region (17% decrease in dropout with a pool of two regions). Similarly, we observed that this approach significantly improved our ability to correctly assign clonality to observed mutations, with a 24% drop in clonality assignment error with the addition of a single region to a pool (Figure 1E). When evaluating these same outcomes at the patient level (i.e. proportion of individuals with at least one variant dropped/misclassified), we observed that pooled sequencing with a single additional region would result in a 14% decrease in both the number of patients subject to mutation dropout and the number affected by misattribution of clonality (Supplementary figure 3b, d). Consistent with the rarity of spatially-delimited low-allele-frequency mutations in ccRCC (arising in cancer genes), we observed a negligible number of false-negative mutation calls with a higher number of regions (Figure 2A). No differences were observed between tumor pools of four regions and those with higher numbers when evaluating their reliability when attributing mutation clonality, however, this result was found specific to the tumor type context (Supplementary figure 4a, b).

Importantly, because the financial cost of next-generation sequencing (NGS) assays is dominated by sequencing costs (i.e. related to library size due to depth and breadth) rather than sample processing and genomic material extraction, obtaining DNA from multiple regions and mixing them into a single pseudo-bulk would result in minimal additions to the total cost (Supplementary Figure 5a). Given that our direct pooling approach requires no additional reagents (nor modifications to the computational infrastructure), it occupies a flexible middle ground

Figure 1 | Intratumoral mutational heterogeneity in renal cell carcinoma can be overcome with pooled sequencing. **A.**Variants identified in the TRACERx RCC cohort. The clonal status and proportion of clinically relevant variants are shown (left). Top 10 most commonly-mutated genes in the TRACERx RCC cohort, by clonal status and clinical relevance (right). The numbers at the top represent the number of unique variants identified per gene in the cohort. **B.** Schematic representing the confection of tumor DNA pools. During 'Evolution inference', evolutionary trees are depicted with (right) and without (left) spatial resolution. **C.** Schematic comparing resource requirements and accuracy across different sequencing approaches. MR: conventional multi-regional sequencing, SR: single-region sequencing. **D.** Variant cancer-cell fraction (CCF) identified in three separate tumor regions and its corresponding multiregional DNA pool. Results are shown in percentages relative to the total number of cancer cells in the sample. E. Dropout (left) and clonality misattribution estimates (right) from the in silico analysis performed on the TRACERx RCC cohort are shown for conventional and pooled sequencing (red and blue bars, respectively). Average event-level results are shown with their 95% confidence intervals (error bars) calculated across 100 simulations.

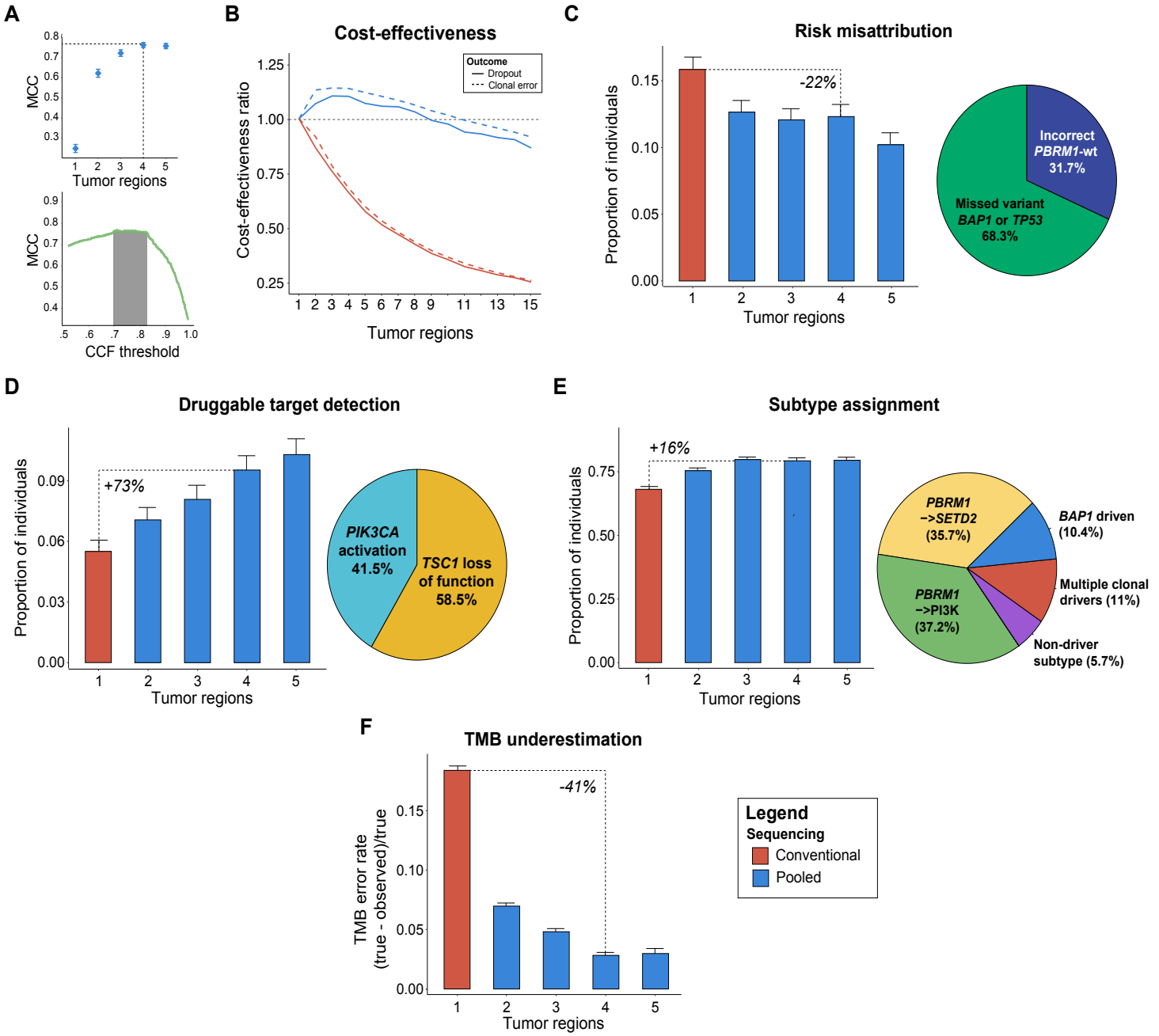

Figure 2 | Translational value of pooled sequencing. A. MCC estimates used to evaluate the optimal number of regions to pool (top) and the optimal range of CCF thresholds to define clonality (bottom). **B.** Cost-effectiveness analysis of the dropout and clonality error rates (at the tumor-level) between conventional and pooled multiregional assessment. Results are shown for increasing numbers of tumor regions relative to the cost-effectiveness of a single tumor region (ratio=1). **C.** Proportion of patients subject to risk misattribution (left). The most-common features resulting in risk misattribution after pooling 4 regions were dropout of BAP1or TP53 variants, followed by dropout of PBRM1 variants (right). **D.** Proportion of patients with at least one targetable mutation identified (left). The most commonly missed targetable alterations in pools of 4 regions were TSC1 loss-of-function and PIK3CA activating mutations (right). **E.** Proportion of patients in which the correct molecular subtype was determined based on mutational data (left). The most commonly missed subtypes in a 4-region tumor pool are shown in the pie chart (right). F. Underestimation of tumor mutational load. Bars represent the average TMB error across all simulations (i.e. true - sample / true) and error bars its 95%CI. TMB: tumor mutational burden, MCC: Matthew's correlation coefficient, CCF: cancer-cell fraction.

compared to bona fide multiregional sequencing and full-scale mixing of left-over tumor tissue[10,11]. We defined a metric of cost-effectiveness as the change in mutation dropout (or clonality) per tumor relative to the change in cost (Supplementary figure 5 b, c). Using cost estimates for targeted panel sequencing from our own institution, we compared the cost-effectiveness of conventional and pooled multi-regional sequencing relative to single-region profiling. Pooled sequencing (of 2 to 4 tumor regions) was found to be ~10% more cost-effective than single-region profiling both for mutation detection and clonality assessment, while the opposite was observed with conventional multi-regional sequencing. Notably, the added benefit of pooled sequencing was lost when pooling 10 regions or more

(Figure 2B).

We next examined the translational utility of pooling discrete regions of a tumor in the management of ccRCC by several metrics. In patients with metastatic ccRCC receiving first-line treatment with tyrosine kinase inhibitors, the mutation status (irrespective of clonal status) of PBRM1, BAP1, and TP53 is of prognostic significance[7], and dropout of somatic variants in these genes therefore affects risk stratification. We observed that risk stratification would be affected in 10% of the TRACERx RCC patients if only a single region were sequenced. Pooled sequencing of 4 regions corrected the risk stratification in 4% of patients, effectively reducing the baseline error in risk stratification by 22% (Figure 2C). Furthermore, the presence of mutations in a subset of genes represents potential therapeutically actionable targets and/or eligibility criteria for clinical trials. Pooled sequencing (of 4 regions) significantly increased the number of patients identified with such mutations by more than 70% (from 6% to 10%) (Figure 2D).

Independent of its translational value, pooled sequencing provides a cost-effective lens onto patterns of ITH. Recent work by the TRACERx consortium in ccRCC has proposed "evolutionary subtypes" based on the presence and clonality of mutations in five genes (VHL, PBRM1, SETD2, BAP1, and PTEN). We examined our capacity to correctly assign evolutionary subtypes in pooled sequencing according to the heuristics outlined by Turajlic and colleagues[6]. Pooling four tumor regions increased the correct evolutionary subtype assignment by 16%, with the majority of missed subtypes corresponding to the 'PBRM1 ➔PI3K' and 'PBRM1➔SETD2' subtypes, with relatively good outcomes (Figure 2E). Pooled sequencing thus represents a potential strategy for the interrogation of subclonal mutational diversity and inference of evolutionary trajectories, which have further implications for patient outcomes.

Finally, we explored the translational value of pooled sequencing in the context of an entirely different disease and sequencing platform. In a cohort of 100 NSCLC patients from the TRACERx consortium, we evaluated in silico the utility of pooled sequencing in accurately quantifying tumor mutation burden (TMB); this measure is employed as a biomarker for response to immunotherapy in this disease. While single region sequencing underestimates total tumor mutation burden by nearly 20%, the addition of a single region to a DNA pool reduced this effect by 41% (Figure 2F). Since the clonality of neoantigens is an emerging determinant of T cell immunoreactivity[13], and given that TMB and neoantigen load are highly correlated[14], accurate assessment of mutation burden with multiregional approaches may improve prognostication in the context of immunotherapy for NSCLC. However, the ability of a clonality-aware TMB measure to predict response to immune-checkpoint blockade will need to be evaluated in this context, as it is currently optimized to the single region setting[15].

Intratumoral heterogeneity is a fundamental hurdle in the genomically-informed delivery of care to cancer patients. In ccRCC, such heterogeneity is so pervasive that it confounds the accurate identification of the small set of driver mutations of therapeutic relevance. Our proposed approach of direct pooled DNA sequencing from several tumor regions overcomes some of these issues at a fraction of the cost of bona fide multiregional profiling; and it does so without excess use of precious tissue material, preserving it for subsequent profiling studies. Pooling thus represents a viable and cost-effective strategy to overcome ITH during clinical sequencing. Importantly, the overhead costs for both single region and pooled sequencing, including sample acquisition, data handling, storage, and analysis, are largely the same, as the size of the sequencing library remains identical. Furthermore, multiregional DNA pooling allows for the inclusion of additional processing steps before sequencing, providing an extra degree of flexibility when adjusting this approach to different clinical scenarios. Finally, by mixing regions of variable purity, we also envision that pooled sequencing may ameliorate the ~3% of tumor samples (~300/10,000 per year total) which currently fail clinical sequencing at our institution due to excessively low tumor purity, thus increasing resource utilization efficiency[16].

Our current analysis is limited to single nucleotide variants and indels. However, copy number variants (CNVs) could be similarly evaluable by pooled sequencing. However, the relatively low density of heterozygous SNPs tiling the genome in targeted sequencing platforms renders the attribution of clonality to CNVs extremely challenging[9]. One might speculate that ongoing refinement of targeted sequencing panels or the use of broader panels could create new opportunities for copy-number analysis from pooled sequencing.

Although sequencing technologies have greatly expanded our knowledge of the molecular mechanisms behind the development and progression of RCC, these discoveries have yet to be translated into tangible clinical benefits during treatment selection or prediction of therapy response. However, it is unclear if this lack of clinical applicability is indeed inherent to the biology of the disease or a result of pervasive sampling biases in past genomic studies that have profiled a single tumor region. Therefore, it is imperative to expand on these initiatives and consider novel sequencing strategies that allow for multi-region assessment of heterogeneous tumors.

SUPPLEMENTAL INFORMATION

Any supplementary information including supplementary figures and supplementary tables, legends, materials and methods, source data and extended data can be found online at https://doi.org/10.52733/KCJ18n2.a1

DATA AVAILABILITY

All the processed data and statistical code needed to reproduce the findings in this study have

been provided in the supplementary materials as well as in a publicly available repository (https://github.com/reznik-lab/DNApooling_RD). The raw sequencing data (MSK-IMPACT) produced in this study are deposited on the Sequence Read Archive (SRA) under the accession number PRJNA633220. Data from the validation sets are available in the supplementary materials of the original TRACERx publications[6,12], only the filtered/annotated versions are provided with this manuscript. Any other relevant data is available from the corresponding authors upon reasonable request.

ACKNOWLEDGMENTS

We thank Charles Swanton, Samra Turajlic, Nicholas McGranahan, Kevin Litchfield and all the members of the TRACERx consortium for facilitating the mutation data used in the study. We thank the Reznik and Chan Lab members for their helpful discussions. We thank the staff and physicians of the MSK Department of Medicine Kidney Program and the Urology Service for their helpful suggestions. We acknowledge the use of the Integrated Genomics Operation Core, funded by the NIH/NCI Cancer Center Support Grant (CCSG, P30 CA008748), Cycle for Survival, and the Marie-Josée and Henry R. Kravis Center for Molecular Oncology. This work was in part supported by grants NIH R35 CA232097 and DOD KC180165 (TAC), as well as the DOD Kidney Cancer Research Program W81XWH-18-1-0318 and the Kidney Cancer Association Young Investigator Award (ER). This work was also supported by the Mellnikoff Fund (TAC) and the Weiss Family Fund (AAH).

COMPETING INTERESTS

T.A.C. is a co-founder of Gritstone Oncology and holds equity. T.A.C. holds equity in An2H. T.A.C. acknowledges grant funding from Bristol-Myers Squibb, AstraZeneca, Illumina, Pfizer, An2H, and Eisai. T.A.C. has served as an advisor for Bristol-Myers, MedImmune, Squibb, Illumina, Eisai, AstraZeneca, and An2H. He also holds ownership of intellectual property on using tumor mutation burden to predict immunotherapy response, with pending patent, which has been licensed to PGDx. The rest of the authors have no conflicts to disclose.

AUTHOR CONTRIBUTIONS

Sample and patient data procurement: AW, AS, YC, PR, JC, AY, BAF
Data processing: VM, RGD, MB, TAC
Statistical analysis: RGD, ED, IO, ER
Manuscript preparation: RGD, RM, NR, ER, AAH
Oversight: AAH, ER, TAC, PR, JH

REFERENCES

1. Hsieh JJ, Purdue MP, Signoretti S, Swanton C, Albiges L, Schmidinger M, et al. Renal cell carcinoma. Nat Rev Dis Primers. 2017;3:17009.
2. Ricketts CJ, Marston Linehan W. Intratumoral heterogeneity in kidney cancer [Internet]. Nature Genetics. 2014. p. 214–5. Available from: http://dx.doi.org/10.1038/ng.2904
3. McGranahan N, Swanton C. Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future. Cell. 2017;168:613–28.
4. Cheng DT, Mitchell TN, Zehir A, Shah RH, Benayed R, Syed A, et al. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): A Hybridization Capture-Based Next-Generation Sequencing Clinical Assay for Solid Tumor Molecular Oncology. J Mol Diagn. 2015;17:251–64.
5. AACR Project GENIE Consortium. AACR Project GENIE: Powering Precision Medicine through an International Consortium. Cancer Discov. 2017;7:818–31.
6. Turajlic S, Xu H, Litchfield K, Rowan A, Horswell S, Chambers T, et al. Deterministic Evolutionary Trajectories Influence Primary Tumor Growth: TRACERx Renal. Cell. 2018;173:595–610.e11.
7. Voss MH, Cheng Y, Marker M, Kuo F, Choueiri TK, Hsieh JJ, et al. Incorporation of PBRM1, BAP1, TP53 mutation status into the Memorial Sloan Kettering Cancer Center (MSKCC) risk model: A genomically annotated tool to improve stratification of patients (pts) with advanced renal cell carcinoma (RCC) [Internet]. Journal of Clinical Oncology. 2018. p. 639–639. Available from: http://dx.doi.org/10.1200/jco.2018.36.6_suppl.639
8. Chakravarty D, Gao J, Phillips SM, Kundra R, Zhang H, Wang J, et al. OncoKB: A Precision Oncology Knowledge Base. JCO Precis Oncol [Internet]. 2017;2017. Available from: http://dx.doi.org/10.1200/PO.17.00011
9. Turajlic S, Sottoriva A, Graham T, Swanton C. Resolving genetic heterogeneity in cancer. Nat Rev Genet. 2019;20:404–16.
10. Zhang X, Garnerone S, Simonetti M, Harbers L, Nicoś M, Mirzazadeh R, et al. CUTseq is a versatile method for preparing multiplexed DNA sequencing libraries from low-input samples. Nat Commun. 2019;10:4732.
11. Litchfield DK, Stanislaw S, Spain L, Gallegos L, Rowan A, Schnidrig D, et al. Representative Sequencing: Unbiased Sampling of Solid Tumor Tissue [Internet]. SSRN Electronic Journal. Available from: http://dx.doi.org/10.2139/ssrn.3404257
12. Jamal-Hanjani M, Wilson GA, McGranahan N, Birkbak NJ, Watkins TBK, Veeriah S, et al. Tracking the Evolution of Non-Small-Cell Lung Cancer. N Engl J Med. 2017;376:2109–21.
13. McGranahan N, Furness AJS, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science. 2016;351:1463–9.
14. Chan TA, Yarchoan M, Jaffee E, Swanton C, Quezada SA, Stenzinger A, et al. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. Ann Oncol. 2019;30:44–56.
15. Budczies J, Allgäuer M, Litchfield K, Rempel E, Christopoulos P, Kazdal D, et al. Optimizing panel-based tumor mutational burden (TMB) measurement. Ann Oncol. 2019;30:1496–506.
16. Zehir A, Benayed R, Shah RH, Syed A, Middha S, Kim HR, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med. 2017;23:703–13.
17. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet. 2011;43:491–8.
18. Kim S, Scheffler K, Halpern AL, Bekritsky MA, Noh E, Källberg M, et al. Strelka2: Fast and accurate variant calling for clinical sequencing applications [Internet]. Available from: http://dx.doi.org/10.1101/192872
19. Koboldt DC, Zhang Q, Larson DE, Shen D, McLellan MD, Lin L, et al. VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res. 2012;22:568–76.
20. Rimmer A, Phan H, Mathieson I, Iqbal Z, Twigg SRF, WGS500 Consortium, et al. Integrating mapping-, assembly- and haplotype-based approaches for calling variants in clinical sequencing applications. Nat Genet. 2014;46:912–8.
21. Sherry ST. dbSNP: the NCBI database of genetic variation [Internet]. Nucleic Acids Research. 2001. p. 308–11. Available from: http://dx.doi.org/10.1093/nar/29.1.308
22. Consortium T 1000 GP, The 1000 Genomes Project Consortium. A map of human genome variation from population-scale sequencing [Internet]. Nature. 2010. p. 1061–73. Available from: http://dx.doi.org/10.1038/nature09534
23. Thorvaldsdottir H, Robinson JT, Mesirov JP. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration [Internet]. Briefings in Bioinformatics. 2013. p. 178–92. Available from: http://dx.doi.org/10.1093/bib/bbs017
24. Barnell EK, Ronning P, Campbell KM, Krysiak K, Ainscough BJ, Sheta LM, et al. Standard operating procedure for somatic variant refinement of sequencing data with paired tumor and normal samples. Genet Med. 2019;21:972–81.
25. Shen R, Seshan VE. FACETS: allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing. Nucleic Acids Res. 2016;44:e131.
26. Bielski CM, Zehir A, Penson AV, Donoghue MTA, Chatila W, Armenia J, et al. Genome doubling shapes the evolution and prognosis of advanced cancers. Nat Genet. 2018;50:1189–95.
27. Jiang Y, Qiu Y, Minn AJ, Zhang NR. Assessing intratumor heterogeneity and tracking longitudinal and spatial clonal evolutionary history by next-generation sequencing. Proc Natl Acad Sci U S A. 2016;113:E5528–37.
28. Carter SL, Cibulskis K, Helman E, McKenna A, Shen H, Zack T, et al. Absolute quantification of somatic DNA alterations in human cancer. Nat Biotechnol. 2012;30:413–21.
29. McGranahan N, Favero F, de Bruin EC, Birkbak NJ, Szallasi Z, Swanton C. Clonal status of actionable driver events and the timing of mutational processes in cancer evolution. Sci Transl Med. 2015;7:283ra54.
30. Turajlic S, Xu H, Litchfield K, Rowan A, Chambers T, Lopez JI, et al. Tracking Cancer Evolution Reveals Constrained Routes to Metastases: TRACERx Renal. Cell. 2018;173:581–94.e12.
31. Chicco D, Jurman G. The advantages of the Matthews correlation coefficient (MCC) over F1 score and accuracy in binary classification evaluation. BMC Genomics. 2020;21:6.
32. Samstein RM, Lee C-H, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet. 2019;51:202–6.

Changes, Challenges and Opportunities in Cancer Care During the COVID-19 Era and Beyond: Building on Lessons Learned From the Pandemic

Robert J. Motzer, MD[1], Eric A. Jonasch, MD[2], Bradley A. McGregor, MD[3], Robert A. Figlin, MD, FACP[4]

[1] *Memorial Sloan Kettering Cancer Center (MSKCC), Weill Cornell Medical College in New York, New York*
[2] *Department of Genitourinary Medical Oncology, Division of Cancer Medicine, MD Anderson Cancer Center*
[3] *Lank Center for Genitourinary Oncology at Dana Farber Cancer Institute, Harvard Medical School, Boston MA*
[4] *Cedars-Sinai Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Health System, Los Angeles, CA*

https://doi.org/10.52733/KCJ19n2-a2

In this roundtable discussion, nation's leading cancer experts from across the country share their perspectives on current changes, challenges and opportunities for delivering cancer care during the COVID-19 era. The roundtable panel examines long-term implications of the pandemic on the management and treatment of cancer especially focusing on significant issues in patient safety, toxicities associated with the use of immunotherapy, COVID-19 vaccination, and clinical trial designs. In this discussion, experts also brainstorm a range of recommendations focusing especially on how the cancer community can capitalize on lessons learned from the pandemic to develop creative approaches that can be taken forward.

INTRODUCTION

The COVID-19 pandemic has caused unprecedented disruption across the spectrum of cancer care services, including cancer diagnosis, screening, clinical trials, and therapeutic management[1-7]. Since the COVID-19 outbreak, substantial decrease in launching new cancer trials or discontinuation of existing trials disrupted the pace of clinical research and new drug discovery with long-term negative consequences for cancer care[2-5]. At the outset of the ongoing COVID-19 crisis, the cancer community is adapting to the substantial challenges that the pandemic continues to pose[7,8]. The lessons learned by the cancer community in the wake of the COVID-19 pandemic offers an opportunity to reflect on the significant issues for delivering optimal cancer care and cancer treatment[9-11]. Interestingly, this outbreak has stimulated innovative approaches in cancer care in an unprecedented way[12]. Here, our distinguished panelists shared a year's worth of lessons that could be used to redesign the delivery of a high standard cancer care and the conduct of cancer clinical trials. The objective of this roundtable program is to gain insights into newly developed measures and recommendations for the cancer community to overcome the long-term impact of the outbreak.

Roundtable questions were distributed to panelists a week before the roundtable session, and the discussion was video recorded and transcribed. The following is a transcript of the roundtable edited for clarity.

Dr. Figlin: This is the Kidney Cancer Journey roundtable focusing on challenges and opportunities in the cancer clinical trials in the COVID-19 era. The purpose of today's roundtable is to gain your insights into how we are all thinking about delivering GU cancer care during the post COVID-19 pandemic world.

I am Robert A. Figlin, *Editor-in-Chief* of the *Kidney Cancer Journal* and the Spielberg family chair at Cedars Sinai Center in Los Angeles. I am really very happy to be joined by three distinguished kidney cancer and GU investigators from across the country. Joining me today are my colleagues Dr. Robert Motzer, the Jack and Dorothy Byrne Chair in Clinical Oncology at Memorial Sloan Kettering Cancer Center (MSKCC) and Professor of Medicine at Weill Cornell Medical College in New York, Head of the Genitourinary Oncology Service. Dr. Eric Jonasch, Professor, Department of Genitourinary Medical Oncology, Division of Cancer Medicine, Director, VHL Clinical Center, MD Anderson Cancer Center and Co-Chair, Renal Cancer Program, MD Anderson Cancer Center and Dr. Bradley McGregor, Clinical director for the Lank Center for Genitourinary Oncology at Dana Farber Cancer Institute, Professor of Medicine, Harvard Medical School.

Correspondence: Robert A. Figlin, MD., FACP., Cedars-Sinai Medical Center, 8700 Beverly Blvd. Los Angeles, CA. 90048. E-mail: robert.figlin@cshs.org

Gentlemen, thank you for taking time out of your busy schedules to join us today.

Bob, let me start out with you. Can you share your view on the role of delivering immunotherapy to kidney cancer patient population in the COVID-19 era?

Dr. Motzer: The COVID-19 pandemic really impacted our practice in many different ways. For one, this pandemic made it difficult for patients to access cancer care and to evaluate patients in our cancer center in Manhattan. This pandemic also made it difficult for healthcare providers to deliver timely cancer therapy to RCC patients. In terms of access to care, patients were screened coming into our clinic and patients were also concerned about coming into Manhattan with the COVID-19 and many times patients would postpone the standard follow-up tests or put them on hold. In return for that, we boosted our remote medical care using videos and so forth. Remote healthcare has been a real challenge. Because it is really new to us and for the most part we have all been practicing hands-on in-person visits. It really forced us to become familiar with these technical aspects.

It is also been a challenge in terms of managing people with metastatic kidney cancer who are on therapies. Because for the most part in the past, you gain so much from seeing somebody in the clinic and talking to them and getting their blood work done rather than speaking with them virtually and not having the comfort level of actually seeing them in person. It also influenced the very close doctor-patient relationship which is very important for a medical oncologist. This pandemic basically put a barrier on that relationship. It also made it difficult for clinical trials. I think that the number of patients going into clinical trials decreased during the pandemic. Patients did not want to come into centers to get into clinical trials as there was more of a concern about experimental medicines and their side effects and also about the impact of the COVID-19 infection. It also made it much more difficult to assess patients from a safety standpoint, because a lot of our safety assessments were compromised by the lack of ability for patients to come and get evaluated. I think it also impacted our treatment to a certain extent for many patients. With the uncertainty around the pandemic and the uncertainty around immunosuppression with the use of steroids, our decision was influenced in terms of when to start patients on systemic therapy or delay it. So it was really an extreme challenge to manage our population of patients with metastatic kidney cancer during the pandemic.

Dr. Figlin: *Bob, that is a great summary. The bigger question is to Eric at MD Anderson Cancer Center in Houston, should we as an organization establish some more coherent guidelines on how to think about our post-pandemic kidney cancer treatments? What are your thoughts about that?*

Dr. Jonasch: Yes, so a couple of things here Bob. First of all, the current guidelines that have been developed by ASCO and ESMO with regards to the general management of immunotherapies are excellent. We can certainly use them as guideposts on how to treat people with immunotherapy, and how to manage immunotherapy related side effects. Layering in then the specter of COVID-19, number one, what happens to an individual who is on immunotherapy who then gets the COVID-19 infection? How does that affect how we treat that individual? Unfortunately, a number of my patients have been infected with COVID-19 while they have been on active checkpoint antibody therapy. It is difficult to see on an individual or an anecdotal level whether or not they have suffered more. Some data suggests that COVID-19 outcomes in cancer patients are worse than people who are without cancer with COVID-19. So I think it is probably impacted them to some degree. As we then assess these individuals from a response perspective, the next question really would be – does the new lymphadenopathy we see on scans correlate with the recent COVID-19 infection or COVID-19? The answer is possibly yes. While you are looking at an individual who has COVID-19, who has pulmonary metastasis, who has inflammatory changes, you would do a RECIST assessment on that person. If patients are on a clinical trial, it certainly creates additional challenges, but not insurmountable ones. I think the last question really is, with the vaccines that are coming out, should we now not vaccinate individuals because of its potential risk? The data has been a little mixed. But there has been a recent paper that came out in the Lancet Oncology by Waissengrin et al, suggesting that the toxicities associated with IO therapy as a function of treatment after vaccination are not dramatically different. And so when my patients asked me, should they get vaccinated? I say, from a toxicity perspective, it is probably better to have a COVID-19 vaccine. From an efficacy perspective, I think it is still an unanswered question. If they get a COVID-19 vaccination, will this worsen the outcome of IO therapy? We still do not have answers.

Dr. Figlin: *That is a brilliant summary. So Brad in Boston, let's just turn our attention to designing clinical trials. how do we need to be thinking about clinical trial design and placing people who are recently COVID-19 vaccinated in the clinical trials post-pandemic?*

Dr. McGregor: I think that is a great question. I get the radiology reports for patients who are on the trials and they are concerned about increased lymphadenopathy correlated with the recent COVID-19 vaccine. So our radiologists are already putting this in the reports on a routine basis. But how do we interpret such data and put blinded, independent review in perspective? I think it is a difficult question to answer at this point of time. We have these different caveats especially once patients receive the recent COVID-19 vaccine. And we started looking at criteria and

interpreted that it may be related to the COVID-19 vaccine. Because from a clinical standpoint, our radiologists are doing this in trials all the time. So it remains to be seen how we incorporate such aspects into trial design.

Dr. Figlin: Yeah, but just to push you a little bit right now, I agree with that completely. Maybe Bob can weigh in as well. I see most of the trials that Bob, Eric and you have reported, are international trials. So patients are from all over the world where the population is highly heterogenous and patients may not have had the vaccine or have the same imaging evaluation as the Farber has, so how are we going to do these pivot international trials, post COVID-19.

Dr. McGregor: Yes, overall, it is challenging when dealing with international pivot trials. If you especially look at the COVID-19 vaccine-induced lymphadenopathy aspect, often these are not large enough to meet RECIST criteria as a new node. I think that is the fortunate thing. So if you are strictly going by RECIST criteria, you may not actually have 3 or 4 centimeter enlarged nodes from a COVID-19 vaccination in the international clinical trials.

Dr. Figlin: *So I want to stay on this topic just for a little bit. Bob, you have led some of the major international trials in kidney cancer, your thoughts about how the datasets will evolve over the next, six to 24 months, and then how we are going to be interpreting that data and how to navigate that. Any thoughts about that?*

Dr. Motzer: We do not really know what the impact of the pandemic will be on the clinical trial data. I think that has been somewhat industry sponsor-specific and the various sponsors have had their own plan in place to monitor follow-up and safety procedures during the COVID-19 pandemic. So, in terms of the studies that I am involved in, I am not aware that there has been a dramatic impact on safety for these trials. There was a study that I was involved with, where there was an issue in terms of getting imaging follow up on patients because of COVID. I think that is something we have to deal within the trial. But for the big phase-3 trials, we have not seen the data come back yet so we are not aware of the impact that you are speaking of. I have been also intrigued by the adenopathy associated with vaccination. So, what we have been doing is we have been timing the scans around vaccine, because I do not think it is a good idea to have a scan on a patient two days after they have a vaccine. But if you do scans two days before vaccination, it is It will be less of an issue and we have noticed that the adenopathy is really quick to resolve. Certainly, in blinded review, core imaging review, people would not know whether there was a vaccine that was responsible for the lymphadenopathy. So I think it is a difficult situation to resolve in trials.

Dr. Figlin: *It is a wonderful conversation, because I think just from the four of us, it may be that the timing of scan imaging and vaccination is important and should become part of data collection during trials, so that we are at least removing the variables from our evaluation of cancer therapy. So Eric, let me turn to you. We have had patients on immunomodulatory steroids and antibodies when they are getting immunotherapy. So how are you navigating through the conversation with your fellows and your patients about the appropriate role for managing the immune-related adverse events associated with immunotherapy?*

Dr. Jonasch: Yes, I guess the big question is - are we changing how we are doing this for individuals in the COVID era? The answer is no. We are clearly using immunomodulatory agents like steroids, IL-6 antibodies, and TNF antibodies, which can have an impact on immunosuppression. But I think it becomes a competing risk argument, which is more important? - managing the severe side effects from immunotherapy, or dealing with the potential increased risk of infectivity while they are on those treatments. I think you have to manage the toxicities from immunotherapy. You can also manage the risk of exposure by making sure that individuals follow safe practices. Recently CDC has announced that people do not need to wear a mask anymore if they are vaccinated. So, there are relaxations of guidelines at a national level. But I think that for our patients, especially for our vulnerable patients, we should probably continue to urge caution and take a conservative approach to the risk of COVID-19 exposure.

Dr. Figlin: *Brad, I want to change the conversation a little bit for all of us to talk about the impact of telemedicine. Bob made an absolutely critical point that the relationship between the medical oncologist and their patient inside that patient care room is a very intimate setting and that is not exactly captured by the Zoom call we are doing today. So, Brad, my question is how have you incorporated telemedicine, and do you think that there will be increased telemedicine going forward as the pandemic winds down or is that just going to dissipate?*

Dr. McGregor: Good question. I think telemedicine is here to stay. There are certain aspects that patients just really enjoy. We have been able to function with telemedicine to an extent and telemedicine will likely incorporate into medicine going forward. It is important to realize that telemedicine is a nice adjunct to in-person care. I do not think it is a replacement for in-person care if you intersperse it between in-person visits. Occasionally, I use telemedicine for the patients who got scared to come into the clinic but when significant cancer care is required or to make important decisions, I would also ask them to visit the hospital in-person to make sure how the patients are actually doing and get a better sense. So it comes out to a fine line where we need to use telemedicine in conjunction with in-person visits. I think that goes the same for clinical trials as well. As we look at the future of clinical trial designs, we can look to incorporate telemedicine as an adjunct wherever possible but not to the extent that it can completely replace in-person

visits.

Dr. Figlin: *What would be your recommendation for our community doctors who always prefer to have doctor-patient interaction but now have a hard job to deliver long-term VEGF TKI therapy without ever seeing the patient.*

Dr. McGregor: From my standpoint, I see out-front. When I start VEGF TKI and check labs for a patient probably every couple of weeks for the first month or so I feel comfortable doing maybe a telemedicine visit at two weeks. But after a month, I like seeing the patient in-person to assess and bring a blood pressure log. It does not put more onus on the patient, because anyway, the patient has to be in the clinic for other data collection that we would otherwise do in-person. I would be happy to see virtually at two weeks, but then I want to have more of a sense of what's actually going on with direct in-person interaction with patients. So it is actually beneficial for patients to come in. In the end, it just comes down to individual discussion with some patients.

Dr. Figlin: *And Bob, what's been your experience in Manhattan with your large patient population?*

Dr. Motzer: I think telemedicine is really a great tool that we need to incorporate into patient care. What I found is telemedicine is particularly useful for our patients who are getting routine follow up with scans and bloodwork and maybe are cancer free or do not require systemic therapy. For these patients, talking via zoom seems reasonable. But, for people who have significant medical problems, or who are on systemic therapy, physicians really need to be seeing those patients. In addition to just providing medications, another important component that really requires seeing the patients, is that physicians provide a tremendous amount of emotional support. It is much more effective to provide that emotional support and contact in person than doing only on a telephone or on a video screen. In the end, it is individual's preference in terms of the physician and the needs of the patient. Certainly, some would say safety visits for patients on chronic TKIs could be done by zoom. On the other hand, if people have symptoms or pain, or are having a very difficult time with regard to family situations, those patients are better off visiting their doctors in person.

Dr. Figlin: *Eric, so many of our patients who come to see us also see other health care providers, social workers, our dieticians and get supportive care, etc. what's been your experience with telemedicine at your center in Houston?*

Dr. Jonasch: Yes, it is clear that there are some advantages and disadvantages to telemedicine that Bob and Brad have outlined here. The access to other specialties in a timely manner as you pointed out is a downside in telemedicine. Another downside is the lack of access to labs that are done locally so patients may not have the reports in hand when speaking with the physicians. Also, the comprehensive care to some degree gets diminished. However, I would actually venture to say that from a social context perspective, patients still cannot bring their loved ones into the hospital with them. Being able to have a conversation with the patient, their spouse, and their children on Zoom without the need for wearing masks in their home environment is great. There are some advantages as patients find that it is more of a conversation with a friend on Zoom than it is a conversation with a doctor. We could improvise telemedicine approaches, for example, incorporate wearables to get vitals on the patient. Apple Watch 4.0 will be able to provide that information to us prior to us actually seeing the patient or while we are seeing the patient. Incorporating these elements into telemedicine and intercalating them with the in-person visits is the future. And I sincerely hope that medical authorities around the country will see that as being an advantage.

Dr. Figlin: *Brad, I would like to get a sense from the three of you whether you have seen patients presenting with a bit more advanced disease, because of maybe not seeing the physician early enough due to COVID-19. And we all get many of our referrals from our surgical colleagues or urologists. What are your thoughts about that? I mean, we do not necessarily need to speak about prostate cancer, where that is happening as well, or bladder cancer, but is there a clinical substantive delay in care that we are starting to experience and worry about in the kinds of patients that you are seeing?*

Dr. McGregor: That is a great question. I think from a GU cancer standpoint, it is more challenging because if you look at something like kidney cancer, bladder cancer, there's no screening in place. So patients often present with symptoms of disease, or we find kidney cancer because they have appendicitis incidentally. I have not seen patients in my clinic who have had hematuria that they have ignored for three months because of the pandemic. Generally, they still see their primary care doctor and get forwarded to the urologist with that workup. So far, I have not seen such a delay in screening but I think time will tell us how this changes with GU cancer people getting less PSA screening or less follow up due to COVID-19 related issues.

Dr. Figlin: *Bob, I know at Cedars during the height of the pandemic, we were having hundreds and hundreds of patients in the hospital with COVID-19, we had a postponement of elective and non-elective surgeries. What's been the experience at MSK?*

Dr. Motzer: that is exactly the same situation we had at MSKCC where during the worst part of the pandemic, a lot of the operating rooms were actually turned into ICU rooms with respirators for COVID patients and a lot of the surgeries that were considered non-emergent were not undertaken or postponed. It was a very difficult situation for patients who had a kidney tumor waiting for their nephrectomy. That is another

aspect in terms of how the pandemic has affected us. I agree with Brad's point. Now, I have not actually noticed in person that patients with kidney cancer presenting to me with a delayed diagnosis because of COVID-19. It certainly seems like a reasonable trend, but I have not observed that with people with GU cancer yet.

Dr. Figlin: *Over the last 15 years, the advancements that we have made in kidney cancer have been a direct result of aggressively placing patients on trials, asking important pivotal questions, and moving therapeutics to the system in a way that is unprecedented in our disease that we all manage. How do you think as a leader in this area that the future clinical trial, whether it is IITs or industry-sponsored research or novel immunotherapy should be designed?*

Dr. Jonasch: Yes, we clearly need to improve. Especially, we need to take lessons from the COVID-19 pandemic in terms of how we can make clinical trials more efficient and how we design our next generation of clinical trials. I am the head of the kidney cancer research consortium of DOD grant-funded mechanism that allows us to try to use informatics solutions to create efficiencies in clinical trial design and execution. I think broadly speaking as a field, we need to embrace such efficient strategies. It is challenging for patients to undergo treatment on a clinical trial with logistical issues. It is a sacrifice that patients are making on behalf of future cancer patients and we have to make that sacrifice as minimal as possible. We may want to add other strategies such as virtual visits, remote monitoring in design so that clinical trials can get less expensive, safer, quicker, and more efficient. So overall, it is going to be a win-win situation.

Dr. Figlin: *So Brad, let me ask you, What are your thoughts if you were to design a 1000 patient international clinical trial tomorrow in kidney cancer, will you be stratifying patients based on COVID-19 infection to ensure the arms are balanced and we are really evaluating the effects of treatment, not the comorbidities that the patients are undergoing?*

Dr. McGregor: It would be very challenging to do that going forward. The challenge is that how to find out if patients had COVID-19 or not in a certain area? And, it is difficult to assess if the side effect comes from the vaccine or from the cancer therapy? These important questions remain to be answered. So, for the clinical trial design, I would include the evaluation of the vaccine efficacy in patients who are on systemic therapy for cancer immunotherapy or chemotherapy. Also, I would be looking to see the antibody titers and how well the vaccines affect side effects overall. Until we really know how to assess COVID-19 and how it affects patients, it becomes very difficult to design a trial stratified by COVID-19 infection criteria.

Dr. Figlin: *I think we all do not want to see the COVID epidemic postpone or delay what we accomplished these years in the kidney cancer space? How are you thinking about that when you are meeting with either your junior investigators or your faculty that are under your guidance? How would you want us to be thinking about that going forward?*

Dr. Motzer: From one point, it is important to minimize the impact of COVID-19. So I recommend that patients who are not only in the US but also outside the United States get the vaccination. As Eric mentioned, the other point is to ensure patients who are treated in the clinical trials get adequate protection including wearing a mask to protect themselves as best as possible. We certainly do not want to see the COVID pandemic truncate our progress in kidney cancer. And so I think along those lines, it is important to discuss the impact of COVID in forums like this so that people are aware of COVID-19 and its potential impact on clinical trials so that we can conduct clinical trials in the setting of the pandemic. Conducting safety evaluations with telemedicine minimizes patients coming into centers and getting exposed. That is been very effective in United States, it would be a wonderful thing if it could be implemented outside of the the United States. We really have not seen a detrimental effect of either the vaccination or the impact of COVID-19 on immunotherapy response. It certainly has been a distraction but I do not think it is a reason to stop clinical trials. Such efforts should help us focus to continue our efforts in clinical trials in RCC.

Dr. Figlin: *Eric, in the Department of Defense and in the funding of clinical trials, are there special questions that we should be asking in the context of the post-pandemic?*

Dr. Jonasch: I think the two key questions we want to answer first are does treatment with immunotherapy worsen side effects from immunotherapy? The second question is, does having COVID or a vaccination alter the ability to respond to treatment? We have an opportunity probably with some of the trials that were winding down as the pandemic occurred. We have to do some retrospective analyses to see whether or not we can tease some of these important questions out. Moving forward, it is going to be a question of what is going to be the issue with COVID. Are we actually going to have this controlled at a national level? Will we have it controlled at an international level?

Dr. Figlin: Brad, as the clinical director of the Lank Center, a very important center nationally for GU studies at the Farber how are you taking the macro view as an institution to contribute to this effort?

Dr. McGregor: I think when COVID first happened in March of 2020, everything came to a screeching halt for about a month. But then, we quickly sort of started coming back and started taking samples as we started adapting to the system and tried to incorporate

those things. And overall, I would say like our clinical operations standpoint, therapies now are very close to where they were pre COVID with telemedicine as an adjunct. Even throughout the pandemic, we have seen clinical trials have been successful in accruing rapidly, Therefore, well designed smart trials can continue to thrive in this setting.

Dr. Figlin: Well, gentlemen, it is been a pleasure hearing your thoughts. It is always nice to see friends and colleagues. And I think our community will welcome as Bob mentioned, conversations like this and can start to inform both how we think about it as well as how we should think about it going forward, especially in the context of not losing the momentum, to address this difficult disease during COVID-19 era, and still wanting to accomplish all the things that we can still accomplish in this setting.

CONCLUDING REMARKS

As we move into the next stage of the pandemic, the biggest challenge is to rapidly adapt to much needed reforms and effectively translate emerging evidence into best practices in delivering a high standard cancer care and treatment. In this discussion, experts reinforced that although the current emphasis is on the management of oncology services, the cancer community should work towards bringing a fresh momentum to quality of care and clinical research in the era of COVID-19 and beyond.

Contributions

The roundtable panelists (authors) were invited to participate in this discussion by the journal. All authors listed in the manuscript contributed significantly to KCJ roundtable. All authors have read and approved the final version. The final content and article is the sole work of the authors.

Acknowledgments

This roundtable program is supported in part by Bristol-Myers Squibb. BMSStudyConnect.com/GUcancers

Declaration of interests

RJM reports grants and other from Bristol Myers Squibb during the conduct of the study; grants and personal fees from Pfizer, grants and personal fees from Novartis, grants and personal fees from Eisai, grants and personal fees from Exelixis, personal fees from Merck, grants and personal fees from Genentech/Roche, personal fees from Lilly, personal fees from Incyte, and personal fees from EMD Serono Research and Development Institute outside the submitted work. *EAJ*: consulting/advisory relationship/ Honoraria received - Exelixis, Merck, Pfizer, Eisai, Novartis, Roche. Research funding - Exelixis, Merck, Pfizer. *BAM*: consultancy for Bayer, Astellas, Astra Zeneca, Seattle Genetics, Exelixis, Nektar, Pfizer, Janssen, Genentech and EMD Serono. Received research support to Dana Farber Cancer Institute (DFCI) from Bristol Myers Squibb, Calithera, Exelixis, Seattle Genetics. *RAF:* no relevant financial relationships with commercial interest to disclose pertaining to this article.

ONLINE CONTENT

Full online contents with additional information available at https://doi.org/10.52733/KCJ19n2-a2

REFERENCES

1. Richards M, Anderson M, Carter P, et al: The impact of the COVID-19 pandemic on cancer care. Nat Cancer 1:565-567, 2020

2. Mahase E. Covid-19: urgent cancer referrals fall by 60%, showing "brutal" impact of pandemic. BMJ 2020; 369: m2386

3. Lamont EB, Diamond SS, Katriel RG, et al. Trends in Oncology Clinical Trials Launched Before and During the COVID-19 Pandemic. JAMA Netw Open. 2021;4(1):e2036353. doi:10.1001/jamanetworkopen.2020.36353

4. Kaufman HW, Chen Z, Niles J, Fesko Y. Changes in the Number of US Patients With Newly Identified Cancer Before and During the Coronavirus Disease 2019 (COVID-19) Pandemic. JAMA Netw Open. 2020;3(8):e2017267. doi:10.1001/jamanetworkopen.2020.17267

5. Kuderer NM, Choueiri TK, Shah DP, et al: Clinical impact of COVID-19 on patients with cancer (CCC19): A cohort study. Lancet 395:1907-1918, 2020

6. Rubinstein SM, Steinharter JA, Warner J, et al: The COVID-19 and Cancer Consortium: A collaborative effort to understand the effects of COVID-19 on patients with cancer. Cancer Cell 37:738-741, 2020

7. US Food and Drug Administration: Clinical Trial Conduct During the COVID-19 Pandemic. https://www.fda.gov/drugs/coronavirus-covid-19-drugs/clinical-trial-conduct-during-covid-19-pandemic

8. Burki TK: Cancer guidelines during the COVID-19 pandemic. Lancet Oncol 21:629-630, 2020

9. Schrag D, Hershman DL, Basch E: Oncology practice during the COVID-19 pandemic. JAMA 323:2005-2006, 2020.

10. Hollander JE, Carr BG: Virtually perfect? Telemedicine for covid-19. N Engl J Med 382:1679-1681, 2020

11. Bailey C, Black JRM, Swanton C: Cancer research: The lessons to learn from COVID-19. Cancer Discov 10:1263-1266, 2020

12. Waterhouse DM, Harvey RD, Hurley P, et al. Early impact of COVID-19 on the conduct of oncology clinical trials and long-term opportunities for transformation: findings from an American Society of Clinical Oncology Survey. JCO Oncol Pract. 2020; 16: 417- 421. doi:10.1200/OP.20.00275

Renal Cell Carcinoma Associated with Germline Mutations in the Krebs Cycle

Eric Lu[1], Brian Shuch[2], Alexandra Drakaki[1,2]

[1] *Division of Hematology/Oncology, University of California, Los Angeles, Los Angeles, CA, USA*

https://doi.org/10.52733/KCJ19n2-a2

ABSTRACT

Germline mutations in the fumarate hydratase (*FH*) and succinate dehydrogenase (*SDH*) genes lead to hereditary leiomyomatosis and RCC (HLRCC) and hereditary paraganglioma and pheochromocytoma, respectively. The renal cell carcinomas that arise in these conditions are characterized by dysregulated Krebs cycles, accumulation of oncometabolites, downstream changes in gene expression, and epigenetic modifications that carry unique therapeutic implications. In this review, we evaluate the current literature on these tumors, including the epidemiology, clinical course, screening guidelines, and management of localized and metastatic disease.

KEYWORDS: Krebs Cycle • Germline Mutations • Oncometabolites • Renal Cell Carcinoma • HLRCC • Papillary RCC

INTRODUCTION

Renal cell carcinoma (RCC) is one of the most common cancers in the United States, with approximately 72,000 new cases and 13,000 deaths projected for 2021.[1] While most RCC occurs sporadically, there is an increasing recognition of the hereditary component for a subset of patients. It is believed that approximately 5-8% of RCC has a strong hereditary component; however, heritability is complex, and kidney cancer genetic predisposition may extend far beyond monogenetic diseases.[2,3] Current estimates, while likely biased, do reinforce that these estimates are not too far off; for instance, large studies such as The Cancer Genome Atlas Program (TCGA) have reported that clear cell, papillary, and chromophobe RCC are associated with germline pathogenic mutations in 6%, 9%, and 6% of individuals respectively.[4] Single-institution series have reported higher numbers of pathogenic germline mutations (up to 16%) with further enrichment in non-clear cell variants. However, over two-thirds of these mutations did not occur in classic RCC genes, suggesting that they are potentially unrelated or, alternatively, that we have yet to characterize the full spectrum of kidney cancer predisposition.[5]

Several autosomal dominant RCC syndromes and associated germline mutations have been well-described, including von Hippel-Lindau (*VHL* gene), hereditary papillary renal cell carcinoma (*MET* gene), Birt-Hogg-Dube´ (*FLCN* gene), hereditary leiomyomatosis and RCC (*FH* gene), succinate dehydrogenase (SDH) deficient kidney cancer (*SDHA, SDHB, SDHC, SDHD* genes), tuberous sclerosis complex (*TSC1* and *TSC2* genes), Cowden syndrome (*PTEN* gene), and microphthalmia-associated transcription factor kidney cancer (*MITF* gene).[6]

Herein, we will focus on hereditary leiomyomatosis and RCC (HLRCC) and SDH-deficient kidney cancer (also known as hereditary paraganglioma and pheochromocytoma), as these two conditions are characterized by Krebs cycle dysfunction, accumulation of oncometabolites, and subsequent predisposition of affected individuals to various malignancies.[6] This link between mitochondrial physiology and tumorigenesis was first observed in the 1920s by Otto Warburg.[7] Accordingly, the genes encoding the Krebs cycle enzymes fumarate hydratase (*FH*) and the succinate dehydrogenase complex (*SDHA, SDHB, SDHC, SDHD* as well as *SDHAF2* which encodes a protein essential for complex assembly) act as tumor suppressors, with germline mutations in these genes leading to accumulation of fumarate and succinate, respectively.[8] These deficits in mitochondrial respiration have been linked to tumorigenesis via several proposed mechanisms. Accumulation of fumarate and succinate as oncometabolites has been shown to inhibit the degradation of HIF1, leading to a state of pseudohypoxia and subsequent downstream gene expression leading to tumorigenesis. Concurrently, excessive fumarate and succinate accumulation leads to DNA and histone methylation, resulting in altered gene expression via epigenetic silencing.[9-11] More recent work has demonstrated that oncometabolite-induced disruption of chromatin signaling leads to an intrinsic homologous recombination DNA repair defect and perhaps further mutational burden. Such findings suggest that FH- and SDH-deficient RCC may represent unique forms of kidney cancer that require different therapeutic approaches.[12]

Correspondence: *Alexandra Drakaki, MD, PhD. Division of Hematology/Oncology and Institute of Urologic Oncology UCLA Health. adrakaki@mednet.ucla.edu*

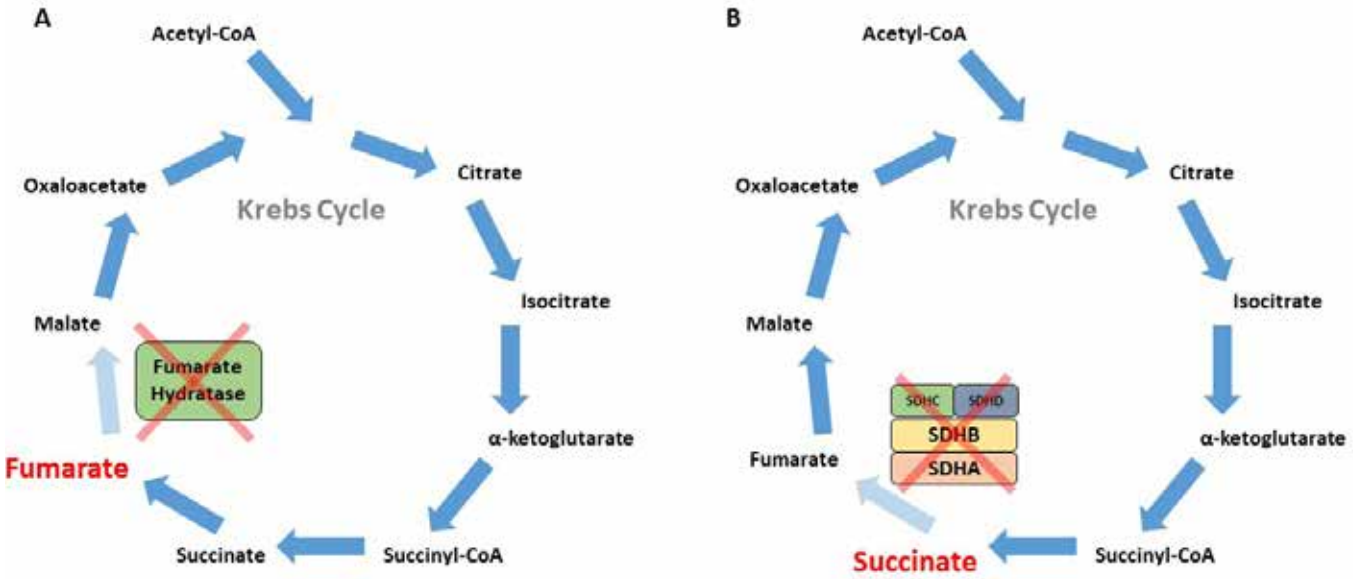

Figure 1 | Krebs Cycle Deficiency and Oncometabolite Accumulation (A) Fumarate hydratase deficiency leads to the accumulation of the oncometabolite fumarate and (B) succinate dehydrogenase deficiency leads to accumulation of the oncometabolite succinate.

HLRCC

The *FH* gene encodes for the Krebs cycle enzyme fumarate hydratase, which catalyzes the conversion of fumarate to malate (**Figure 1A**). The loss of function in the *FH* gene leads to the autosomal dominant cancer syndrome known as HLRCC, which is characterized by cutaneous leiomyomas, early-onset and highly symptomatic uterine leiomyomas, adrenal macronodular hyperplasia, and a very aggressive form of kidney cancer now recognized as its own subtype – FH-deficient kidney cancer. This subtype can resemble papillary type 2, collecting duct, and tubulocystic RCC (**Figure 2**).[13,14] The incidence and prevalence of HLRCC are currently unknown, but now several hundred families have been described in the literature.[15] The prevailing consensus was that HLRCC is quite rare, and among those with pathogenic *FH* mutations, the lifetime cumulative risk of RCC was 15-30%.[16,17] However with the widespread availability of panel testing that included the *FH* gene, many more patients are now being identified, leading to the belief that this condition is under-recognized. With large exome databases available, it is now evident that *FH* alterations are very common with carrier estimates between 1/1000 and 1/2500 individuals, suggesting a much lower RCC penetrance closer to 2-6%.[18]

Renal tumors in HLRCC tend to develop at an earlier age, with one series reporting a median age of onset of 37 years with a range of 10-77.[3] Tumors tend to be unilateral and solitary with a particularly aggressive biological behavior compared to other types of hereditary kidney cancer. Imaging characteristics frequently demonstrate an infiltrative nature (>85%) with the invasion of the renal sinus fat (>80%) (**Figure 2**).[19] Even smaller tumors have a propensity for early and rapid nodal and distant metastasis, as evidenced by one series in which four of seven patients with 2.0-6.7 cm T1 tumors had spread to regional lymph nodes or had distant metastases at the time of nephrectomy.[20] Another study found that among HLRCC patients with RCC, 47% (16/34) were metastatic at diagnosis, and another 35% (12/34) became metastatic within 3 years of diagnosis.[21]

Due to this accelerated growth rate and potential for metastatic spread even with small primary tumors, annual abdominal imaging (MRI favored over CT) is recommended for surveillance starting at age 8-10 for those at risk.[16,22] For localized tumors, surveillance is not recommended, even for smaller tumors <1 cm. Rather, partial nephrectomy with wide surgical margins with consideration of retroperitoneal lymphadenectomy is recommended. Radical nephrectomy could also be considered if partial nephrectomy is not felt to be able to achieve a wide margin.[16] Although enucleation for small RCC tumors has increased in popularity over recent years, for FH-deficient RCC, the surgeon must keep a safe distance away from the tumor as local recurrences are common.

For metastatic HLRCC patients, therapeutic options are unfortunately limited with no accepted standard. Given that oncometabolite (fumarate) accumulation leads to a disruption of the Krebs cycle and a shift towards dependence on aerobic glycolysis for energy needs, these tumors may become uniquely dependent on aerobic glycolysis, which is sustained by high glucose influx. Furthermore, tumorigenesis is driven by the pseudohypoxia pathway, rendering these tumors particularly sensitive to drugs directed against molecular targets downstream of HIF1-alpha. Consequentially, vascular endothelial growth factor receptor (VEGF) pathway inhibitors represent the most rational therapeutic choice over immunotherapy.

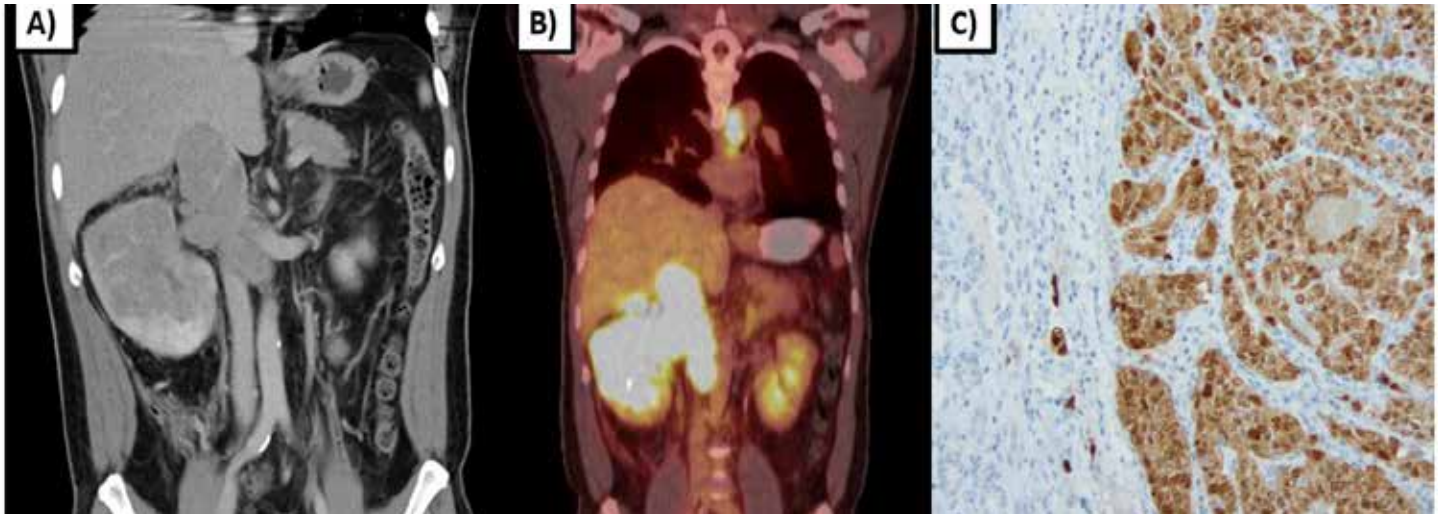

Figure 2 | HLRCC-associated Renal Cell Carcinoma. (A) Contrast-enhanced CT scan (coronal view) showing large renal mass, large tumor thrombus, and retroperitoneal lymphadenopathy. (B) Demonstrated lesion is FDG-PET avid with associated mediastinal FDG-PET avid metastases. (C) Microscopic evaluation (40X) of a renal tumor with papillary-like morphology. FH was not lost however staining with 2S-C demonstrated high cytoplasmic expression and absence in the adjacent normal kidney tissue.

Several ongoing clinical trials are testing rational drug targets in patients with HLRCC-associated renal tumors. The combination of bevacizumab plus erlotinib has shown promising activity in an ongoing phase II clinical trial of patients with papillary RCC, both HLRCC and sporadic (NCT01130519). Results from an abstract published in May of 2020 detail that for the 83 treated patients, the objective response rate was 64% (27/42) in the HLRCC cohort and 37% (15/41) in a sporadic papillary RCC cohort. Median PFS was 21.1 months in the HLRCC cohort and 8.7 months in the sporadic cohort.[23] Most adverse events were grade 1-2, and the most notable grade ≥3 adverse events were hypertension (34%) and proteinuria (13%), as expected from bevacizumab. In addition, one patient died from gastrointestinal hemorrhage possibly attributable to bevacizumab therapy. The investigators postulated that the increased activity among HLRCC patients may be related to FH inactivation resulting in upregulation of HIF, with the resulting metabolic alterations leading the tumors to be uniquely susceptible to this combination.

The responsiveness to this regimen was also observed in a small retrospective series from South Korea where the objective response rate was 50% (5/10).[24] A more recent randomized phase 2 trial, SWOG 1500, established cabozantinib (a dual MET and VEGF inhibitor) as a promising option for patients with papillary RCC. However, given that HLRCC-associated renal tumors (previously classified as papillary type 2) represent a distinct morphologic and molecular subset that is metabolically deficient, rather than MET-driven as with papillary tumors, it remains unclear whether the results from this trial are generalizable to HLRCC patients. Nonetheless, for the small subset of patients in this trial with HLRCC, it will be important to follow the long-term outcomes on cabozantinib.[14,25]

Other strategies involving immunotherapy have been described in case reports. In one instance, the use of axitinib plus the PD-1 blocker sintilimab in a patient with metastatic HLRCC-associated RCC resulted in disease stabilization and improvement of symptoms.[26] In another case, combination immunotherapy with nivolumab plus ipilimumab for metastatic disease led to a complete response with an ongoing durable remission at 68 weeks.[27] However, given that larger studies are lacking, it remains unclear at this time what role immunotherapy plays in the treatment of this disease.[22]

SDH-DEFICIENT RCC

SDH is an enzyme complex that is composed of four subunits (SDHA, SDHB, SDHC, SDHD) and assembled with SDHAF1 and SDHAF2 mitochondrial proteins. This enzyme plays a critical role in mitochondrial respiration; specifically, it catalyzes the oxidation of succinate to fumarate within the Krebs cycle and is also critical to complex 2 of the electron transport chain (**Figure 1B**). Those with germline loss of function mutations in one of these *SDH* genes are at risk for paragangliomas, pheochromocytomas, gastrointestinal stromal tumors (GIST), and RCC.[28]

RCC has been reported in individuals with *SDHB, SDHC*, and *SDHD* mutations, and more recently, in patients with *SDHA* mutations.[29] Histology can be variable and may depend on the subunit affected. *SDHB*-deficient tumors are characterized by a unique oncocytic and vacuolated appearance. By contrast, *SDHC*-deficient tumors tend to present with clear cell histology.[28,30] Various other histologies, including papillary, sarcomatoid, and unclassified RCCs have been reported in patients with germline mutations of *SDH* subunit genes (***Figure 3***).

As opposed to sporadic RCC,

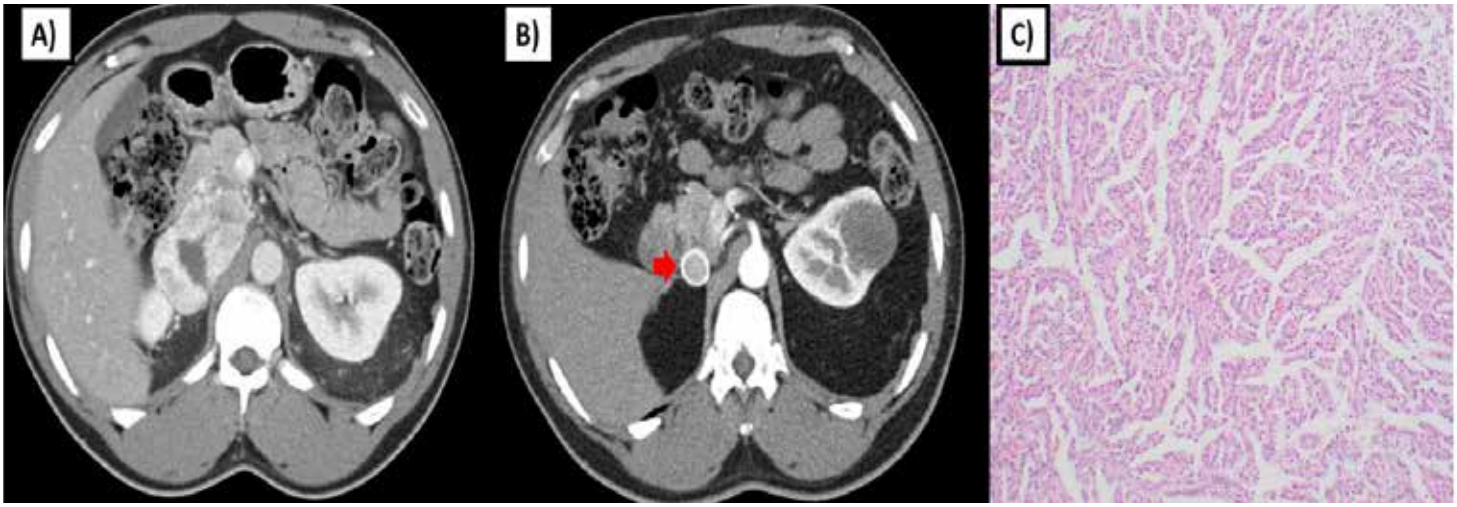

Figure 3 | SDHA-deficient Pheochromocytoma and Renal Cell Carcinoma. (A) A 33-year-old gentleman who presented with a large invasive left pheochromocytoma invading the inferior vena cava in patient with germline pathogenic *SDHA* mutation. (B) Six years later at age 39, the patient developed a 4.5 cm left renal mass. The patient's IVC graft from the pheochromocytoma is shown with a red arrow. (C) The patient had a robotic partial nephrectomy for a pT1b papillary type I RCC with some atypical nuclear characteristics.

SDH-deficient RCC tends to occur at an earlier age with a reported median age of onset 30 years and range from 15 to 61 in one series.[3] Given that these tumors share common underlying metabolic features with FH-deficient RCC, some may behave in a similarly aggressive manner and present with metastatic disease; however, others may have a more low-grade appearance and present with non-invasive tumors (**Figure 3**). Although penetrance for paragangliomas and pheochromocytomas are much higher in those with a germline *SDH* mutation (18-95% by age 60), RCC can also present as the sole finding in such individuals.[28,31] The true penetrance for RCC in SDH-deficiency is unknown and may vary by the affected subunit, but some reports estimate that risk could be up to 14% by age 70.[32] Given the variable histologies and uncertain penetrance of RCC in SDH-deficiency, screening recommendations vary. The National Comprehensive Cancer Network recommends abdominal imaging (MRI or CT) with and without contrast every 4-6 years starting at age 12.[22] However this approach should be individualized based on family risk.

SDH-deficient RCC tends to have a lower risk for metastatic disease but a high incidence of bilateral tumors (with 26% bilateral tumors in one series of 27 patients with prolonged

Genetic Syndrome	Gene Mutation Inheritance	Histology (RCC)	Penetrance (RCC)	Clinical Manifestations	Surveillance Guidelines	1st-line Systemic Therapy for RCC	Specialists Involved
Hereditary leiomyomatosis and renal cell cancer (HLRCC)	*Fumarate Hydratase (FH)* Autosomal dominant	FH-deficient kidney cancer	2-6%[18]	Cutaneous leiomyomas, severe symptomatic uterine leiomyomas, adrenal macronodular hyperplasia	Abdominal MRI with and without contrast annually starting at age 8-10[22]	Bevacizumab plus erlotinib[23]	Dermatology Gynecology Oncology Urology Genetics
Hereditary paraganglioma and pheochromocytoma (SDH-deficient RCC)	*Succinate dehydrogenase* (*SDHA, SDHB, SDHC, SDHD, SDHAF2*) Autosomal dominant	Variable, including clear cell (not typical with *SDHB*), papillary, sarcomatoid, oncocytic neoplasm	Variable depending on subunit[32]	Paragangliomas, pheochromocytomas, GIST tumors	Abdominal MRI with and without contrast every 4-6 years starting at age 12[22]	VEGF inhibitor monotherapy[3] 4-36	Endocrinology Endocrine Surgery Oncology Urology Genetics

Table 1 | Overview of Krebs Cycle Deficient Hereditary Renal Cell Carcinoma

follow-up). Nonetheless, surveillance, even for smaller tumors, is not recommended.[32] For individuals with non-invasive tumors, nephron-sparing surgery can be considered. However, for individuals with higher risk tumors (large, invasive, high-grade, infiltrative), the risk for development of metastatic disease is high, and radical nephrectomy with lymph node dissection should be considered.[33] Patients will require long-term follow-up due to potential for late recurrences, metachronous disease, and other syndromic manifestations of germline SDH deficiency (ie, paraganglioma, pheochromocytomas, GIST).

For metastatic SDH-deficient RCC, there is no widely accepted frontline therapy, as evidence is limited to single case series. Much like HLRCC, given the unique metabolic disturbance caused by oncometabolite (succinate) accumulation, VEGF inhibitors are commonly used. One case of widely metastatic RCC of unclassifiable histology with high-grade features was initially treated with sunitinib with a 15-month duration of response, followed by temsirolimus with only a 2-month duration of response, before ultimately being found to be positive for a *SDHB* mutation. The patient was subsequently treated with pazopanib (a multi-kinase angiogenesis inhibitor) with symptomatic response and stabilization of metastatic disease. However, the patient experienced progression of his cancer 6 months later and ultimately succumbed to the disease.[34] In another report, an individual with *SDHC*-deficient clear cell RCC who eventually developed widespread metastases not amenable to locally directed therapies was treated with sunitinib and remained on therapy for 34 months with a near-complete response.[35] In a third case, an individual with a *SDHA* germline mutation developed a high-grade papillary type 2 RCC with sarcomatoid dedifferentiation that was initially refractory to anti-PD-1 treatment, but later experienced disease stabilization while on a series of VEGF tyrosine kinase inhibitors (sunitinib, pazopanib, and sorafenib).[36]

FUTURE DIRECTIONS

Given limited therapeutic options for patients with advanced Krebs cycle deficient RCC and limited experience using immunotherapy in this setting, novel therapeutic approaches are needed. Recently, HIF-2α has emerged as a druggable target in clear cell RCC. In a phase I/II study with 55 patients with advanced clear cell RCC receiving the oral HIF-2α inhibitor MK-6482, overall response rate was 24% and disease control rate was 80%.[37] Given that HLRCC-related RCC and SDH-deficient RCC are characterized by oncometabolite accumulation and pseudohypoxia via stabilization of HIF1, this may be a promising class of therapy worthy of further study. Concurrently, recent evidence demonstrating that these metabolically deficient tumors are deficient in homologous recombination DNA repair has emerged, suggesting that these tumors may be uniquely susceptible to PARP inhibition. Consequentially, our team is opening an upcoming trial utilizing the PARP inhibitor pamiparib plus temozolomide in patients with HLRCC (NCT04603365).[12]

CONCLUSIONS

Although accounting for a minority of RCC cases, Krebs cycle deficient RCC represents a molecularly distinct subset that is often extremely clinically aggressive. Given the complexities of diagnosis and management of such diseases, a multi-disciplinary approach is critical. For now, early surgical excision is the standard for localized disease, and VEGF-based therapy remains the mainstay for metastatic disease. However, moving forward, the unique metabolic features of Krebs cycle deficient tumors may be amenable to more refined therapeutic targeting, and many such efforts are already underway to improve clinical outcomes.

ONLINE INFORMATION

https://doi.org/10.52733/KCJ19n2-a2

REFERENCES

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. CA: A Cancer Journal for Clinicians 2021;71:7-33.

2. Scelo G, Purdue MP, Brown KM, et al. Genome-wide association study identifies multiple risk loci for renal cell carcinoma. Nat Commun 2017;8:15724.

3. Shuch B, Vourganti S, Ricketts CJ, et al. Defining early-onset kidney cancer: implications for germline and somatic mutation testing and clinical management. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2014;32:431-7.

4. Huang KL, Mashl RJ, Wu Y, et al. Pathogenic Germline Variants in 10,389 Adult Cancers. Cell 2018;173:355-70.e14.

5. Carlo MI, Mukherjee S, Mandelker D, et al. Prevalence of Germline Mutations in Cancer Susceptibility Genes in Patients With Advanced Renal Cell Carcinoma. JAMA Oncol 2018;4:1228-35.

6. Linehan WM, Schmidt LS, Crooks DR, et al. The Metabolic Basis of Kidney Cancer. Cancer Discov 2019;9:1006-21.

7. Warburg O, Wind F, Negelein E. THE METABOLISM OF TUMORS IN THE BODY. J Gen Physiol 1927;8:519-30.

8. Pollard PJ, Brière JJ, Alam NA, et al. Accumulation of Krebs cycle intermediates and over-expression of HIF1alpha in tumours which result from germline FH and SDH mutations. Hum Mol Genet 2005;14:2231-9.

9. Killian JK, Kim SY, Miettinen M, et al. Succinate dehydrogenase mutation underlies global epigenomic divergence in gastrointestinal stromal tumor. Cancer Discov 2013;3:648-57.

10. Morin A, Letouzé E, Gimenez-Roqueplo AP, Favier J. Oncometabolites-driven tumorigenesis: From genetics to targeted therapy. Int J Cancer 2014;135:2237-48.

11. Selak MA, Armour SM, MacKenzie ED, et al. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase.

Cancer Cell 2005;7:77-85.

12. Sulkowski PL, Oeck S, Dow J, et al. Oncometabolites suppress DNA repair by disrupting local chromatin signalling. Nature 2020;582:586-91.

13. Launonen V, Vierimaa O, Kiuru M, et al. Inherited susceptibility to uterine leiomyomas and renal cell cancer. Proc Natl Acad Sci U S A 2001;98:3387-92.

14. Merino MJ, Torres-Cabala C, Pinto P, Linehan WM. The morphologic spectrum of kidney tumors in hereditary leiomyomatosis and renal cell carcinoma (HLRCC) syndrome. Am J Surg Pathol 2007;31:1578-85.

15. Patel VM, Handler MZ, Schwartz RA, Lambert WC. Hereditary leiomyomatosis and renal cell cancer syndrome: An update and review. J Am Acad Dermatol 2017;77:149-58.

16. Menko FH, Maher ER, Schmidt LS, et al. Hereditary leiomyomatosis and renal cell cancer (HLRCC): renal cancer risk, surveillance and treatment. Fam Cancer 2014;13:637-44.

17. Wei MH, Toure O, Glenn GM, et al. Novel mutations in FH and expansion of the spectrum of phenotypes expressed in families with hereditary leiomyomatosis and renal cell cancer. J Med Genet 2006;43:18-27.

18. Shuch B, Li S, Risch H, Bindra RS, McGillivray PD, Gerstein M. Estimation of the carrier frequency of fumarate hydratase alterations and implications for kidney cancer risk in hereditary leiomyomatosis and renal cancer. Cancer 2020;126:3657-66.

19. Nikolovski I, Carlo MI, Chen YB, Vargas HA. Imaging features of fumarate hydratase-deficient renal cell carcinomas: a retrospective study. Cancer Imaging 2021;21:24.

20. Grubb RL, 3rd, Franks ME, Toro J, et al. Hereditary leiomyomatosis and renal cell cancer: a syndrome associated with an aggressive form of inherited renal cancer. J Urol 2007;177:2074-9; discussion 9-80.

21. Muller M, Ferlicot S, Guillaud-Bataille M, et al. Reassessing the clinical spectrum associated with hereditary leiomyomatosis and renal cell carcinoma syndrome in French FH mutation carriers. Clin Genet 2017;92:606-15.

22. NCCN Clinical Practice Guidelines in Oncology - Kidney Cancer. 2021. at https://www.nccn.org/professionals/physician_gls/pdf/kidney.pdf.)

23. Srinivasan R, Gurram S, Harthy MA, et al. Results from a phase II study of bevacizumab and erlotinib in subjects with advanced hereditary leiomyomatosis and renal cell cancer (HLRCC) or sporadic papillary renal cell cancer. Journal of Clinical Oncology 2020;38:5004-.

24. Choi Y, Keam B, Kim M, et al. Bevacizumab Plus Erlotinib Combination Therapy for Advanced Hereditary Leiomyomatosis and Renal Cell Carcinoma-Associated Renal Cell Carcinoma: A Multicenter Retrospective Analysis in Korean Patients. Cancer Res Treat 2019;51:1549-56.

25. Pal SK, Tangen C, Thompson IM, et al. A comparison of sunitinib with cabozantinib, crizotinib, and savolitinib for treatment of advanced papillary renal cell carcinoma: a randomised, open-label, phase 2 trial. The Lancet 2021;397:695-703.

26. Feng D, Yang Y, Han P, Wei X. The preliminary outcome of the combination of immunotherapy and targeted therapy after recurrence and metastasis for hereditary leiomyomatosis and renal cell cancer-a case report. Transl Androl Urol 2020;9:789-93.

27. Iribe Y, Furuya M, Shibata Y, et al. Complete response of hereditary leiomyomatosis and renal cell cancer (HLRCC)-associated renal cell carcinoma to nivolumab and ipilimumab combination immunotherapy by: a case report. Fam Cancer 2021;20:75-80.

28. Ricketts CJ, Shuch B, Vocke CD, et al. Succinate dehydrogenase kidney cancer: an aggressive example of the Warburg effect in cancer. J Urol 2012;188:2063-71.

29. Yakirevich E, Ali SM, Mega A, et al. A Novel SDHA-deficient Renal Cell Carcinoma Revealed by Comprehensive Genomic Profiling. Am J Surg Pathol 2015;39:858-63.

30. Gupta S, Swanson AA, Chen YB, et al. Incidence of succinate dehydrogenase and fumarate hydratase-deficient renal cell carcinoma based on immunohistochemical screening with SDHA/SDHB and FH/2SC. Hum Pathol 2019;91:114-22.

31. Eijkelenkamp K, Osinga TE, de Jong MM, et al. Calculating the optimal surveillance for head and neck paraganglioma in SDHB-mutation carriers. Fam Cancer 2017;16:123-30.

32. Ricketts CJ, Forman JR, Rattenberry E, et al. Tumor risks and genotype-phenotype-proteotype analysis in 358 patients with germline mutations in SDHB and SDHD. Hum Mutat 2010;31:41-51.

33. Gill AJ, Hes O, Papathomas T, et al. Succinate dehydrogenase (SDH)-deficient renal carcinoma: a morphologically distinct entity: a clinicopathologic series of 36 tumors from 27 patients. Am J Surg Pathol 2014;38:1588-602.

34. Paik JY, Toon CW, Benn DE, et al. Renal carcinoma associated with succinate dehydrogenase B mutation: a new and unique subtype of renal carcinoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2014;32:e10-3.

35. Shuch B, Agochukwu N, Ricketts CJ, et al. Vascular Endothelial Growth Factor Receptor-Targeted Therapy in Succinate Dehydrogenase C Kidney Cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2016;34:e76-9.

36. McEvoy CR, Koe L, Choong DY, et al. SDH-deficient renal cell carcinoma associated with biallelic mutation in succinate dehydrogenase A: comprehensive genetic profiling and its relation to therapy response. NPJ Precis Oncol 2018;2:9.

37. Choueiri TK, Plimack ER, Bauer TM, et al. Phase I/II study of the oral HIF-2 α inhibitor MK-6482 in patients with advanced clear cell renal cell carcinoma (RCC). Journal of Clinical Oncology 2020;38:611-.

ASCO21 Kidney Cancer Roundup

https://doi.org/10.52733/KCJ19n2-e1

Robert A Figlin, MD

The COVID-19 pandemic has exposed fundamental disparities in the provision of health care across our nation and exacerbated the differences in health outcomes associated with race, socioeconomic and other demographic factors. A silver lining however is that pandemic precarity has inspired tremendous scientific collaboration among clinicians, researchers, and key opinion leaders. In this line, this year's Annual Meeting of the American Society of Clinical Oncology (ASCO21) which was kicked off virtually on June 4 through June 8, not only celebrated latest breakthroughs in cancer research, treatment and patient care, but also focused on health equity in cancer care. ASCO21's fitting theme - Health equity "doing right by the patients for whom we care" reflected addressing complex forces and systems that have created disparities in cancer care, treatment, and research and identifying ways to ensure that all patients have access to and benefit from the latest cancer advances and high-quality cancer care.

Let's recap the key developments from some of the highest-profile clinical trials presented in the ASCO21 conference. The results from KEYNOTE-564 trial presented during plenary session of ASCO21 have demonstrated that post-surgery treatment with pembrolizumab extends DFS for clear cell renal cell carcinoma (RCC) patients. This is the first phase III study based on adjuvant immunotherapy to demonstrate an improvement in DFS with a favorable safety profile as compared to S-TRAC and sunitinib. KEYNOTE-564's findings support adjuvant pembrolizumab as a potential new standard of care to reduce disease recurrence in patients with fully resected intermediate to high-risk RCC. However, further works remains to be seen especially confirmation of an overall survival benefit, usability in other RCC histologies, and the implications regarding treatment choices in advanced RCC. Additionally the trial included M1 patients post treatment of the oligometastatic disease. This is not a usual cohort of patinets included in classical adjuvant trials and may affect the DFS reported. Currently, there are four IO combinations available for the first-line treatment of metastatic RCC viz. nivolumab plus ipilimumab, pembrolizumab plus axitinib, avelumab plus axitinib, and nivolumab plus cabozantinib. The latest outcome of CLEAR trial adding lenvatinib plus pembrolizumab combination to the growing armamentarium of first-line treatments for RCC patients. CLEAR met its primary endpoint, with lenvatinib plus pembrolizumab significantly improving progression-free survival compared to sunitinib. This combination also achieved favorable objective response rate compared to sunitinib with an impressive complete response rate. Lenvatinib + everolimus combination resulted in similar or worse HRQoL and symptom scores compared with patients treated with sunitinib. The additional data builds on previous findings of a phase 3 KEYNOTE-581 trial has shown (Abstract 4502); PD-1 inhibitor pembrolizumab plus lenvatinib improved outcomes versus sutent on a measure of health-related quality of life in first-line renal cell carcinoma (RCC). The additional data showed an improvement of specific health-related quality of life measures including favorable disease-related symptoms scores, as well as better HRQoL and disease-related symptoms scores for physical functioning, fatigue, dyspnea and constipation versus sutent.

Results from KEYNOTE-426's prespecified final analysis with a 42.8-month median follow-up and a 35.6-month minimum follow-up show that pembrolizumab + axitinib continues to demonstrate superior efficacy over sunitinib with respect to OS, PFS, and objective response rate. KEYNOTE-426 represents the longest follow-up of an anti-PD–1/L1 immunotherapy combined with a VEGF/VEGFR inhibitor for first line RCC and indicate pembrolizumab + axitinib as standard of care for patients with previously untreated advanced clear cell RCC. Results from CANTATA study shows that the addition of telaglenastat did not improve the efficacy of cabozantinib in mRCC as there was no significant difference in PFS between the arms. While the addition of telaglenastat did not improve outcomes with cabozantinib in this unselected population of patients with clear cell RCC, future studies are warranted to determine the impact of glutaminase inhibition in biomarker-selected patient populations with high dependence on glutamine/glutaminase, and in combination with other therapeutic partners. A study involving Cabozantinib plus nivolumab (CaboNivo) combination showed that CaboNivo had an acceptable safety profile and showed promising efficacy in metastatic non-clear cell RCC pts with papillary, unclassified, or translocation associated histologies whereas activity in patients with chromophobe RCC was limited. Latest results from phase 2 study of belzutifan (MK-6482) shown clinical benefit and has a favorable safety profile in patients with VHL disease–associated ccRCC, pNETs, and hemangioblastomas.. In the phase 3 CheckMate (CM) 9ER trial, Nivolumab in combination with cabozantinib (N+C) has demonstrated significantly improved progression-free survival (PFS), objective response rate (ORR), and overall survival (OS), compared with sunitinib as a first-line (1L) treatment for aRCC.

In this issue, an exclusive roundtable discussion which I have moderated, participated by three distinguished kidney cancer and genitourinary investigators from across

the country focusing on the current challenges in cancer trials post COVID-19 pandemic. These renowned experts also brainstormed various important topics including cancer therapy delivery in the COVID-19 era, telemedicine, new COVID-19 guidelines, evolving clinical trial design, vaccination efforts in cancer patients, adverse events associated with vaccination, and other newly developed measures and recommendations to manage COVID-19 issues in the provision of cancer care. In the review work, Eric *et al* evaluated the current literature regarding the epidemiology, clinical course, screening guidelines, and management of localized and metastatic disease of krebs cycle deficient RCCs such as hereditary leiomyomatosis and RCC (HLRCC) and hereditary paraganglioma and pheochromocytoma and also summarized therapeutic targeting strategies germline mutations in the fumarate hydratase (*FH*) and succinate dehydrogenase (*SDH*) genes participating in the krebs cycle metabolism. A seminal work by DiNatale *et al* proposed a pooled multiregional DNA sequencing strategy to boost translational value by addressing ITH while preserving valuable residual tissue for secondary analysis in RCC like solid tumors.

Robert A. Figlin, MD, FACP

KCJ JOURNAL CLUB

Essential Peer-Reviewed Reading

The peer-reviewed articles summarized in this section were selected by the Editor-in-Chief, Robert A. Figlin, MD, for their timeliness, importance, relevance, and potential impact on clinical practice or translational research.

Nivolumab plus Cabozantinib versus Sunitinib for Advanced Renal-Cell Carcinoma. Choueiri TK et al. N Engl J Med . 2021 Mar 4;384(9):829-841. doi: 10.1056/NEJMoa2026982

Methods: In this phase 3, randomized, open-label trial, we randomly assigned adults with previously untreated clear-cell, advanced renal-cell carcinoma to receive either nivolumab (240 mg every 2 weeks) plus cabozantinib (40 mg once daily) or sunitinib (50 mg once daily for 4 weeks of each 6-week cycle). The primary end point was progression-free survival, as determined by blinded independent central review. Secondary end points included overall survival, objective response as determined by independent review, and safety. Health-related quality of life was an exploratory end point.

Results: Overall, 651 patients were assigned to receive nivolumab plus cabozantinib (323 patients) or sunitinib (328 patients). At a median follow-up of 18.1 months for overall survival, the median progression-free survival was 16.6 months (95% confidence interval [CI], 12.5 to 24.9) with nivolumab plus cabozantinib and 8.3 months (95% CI, 7.0 to 9.7) with sunitinib (hazard ratio for disease progression or death, 0.51; 95% CI, 0.41 to 0.64; P<0.001). The probability of overall survival at 12 months was 85.7% (95% CI, 81.3 to 89.1) with nivolumab plus cabozantinib and 75.6% (95% CI, 70.5 to 80.0) with sunitinib (hazard ratio for death, 0.60; 98.89% CI, 0.40 to 0.89; P = 0.001). An objective response occurred in 55.7% of the patients receiving nivolumab plus cabozantinib and in 27.1% of those receiving sunitinib (P<0.001). Efficacy benefits with nivolumab plus cabozantinib were consistent across subgroups. Adverse events of any cause of grade 3 or higher occurred in 75.3% of the 320 patients receiving nivolumab plus cabozantinib and in 70.6% of the 320 patients receiving sunitinib. Overall, 19.7% of the patients in the combination group discontinued at least one of the trial drugs owing to adverse events, and 5.6% discontinued both. Patients reported better health-related quality of life with nivolumab plus cabozantinib than with sunitinib.

Conclusions: Nivolumab plus cabozantinib had significant benefits over sunitinib with respect to progression-free survival, overall survival, and likelihood of response in patients with previously untreated advanced renal-cell carcinoma. (Funded by Bristol Myers Squibb and others; CheckMate 9ER ClinicalTrials.gov number, NCT03141177.).

Lenvatinib plus Pembrolizumab or Everolimus for Advanced Renal Cell Carcinoma. Motzer et al. N Engl J Med. 2021 Apr 8;384(14):1289-1300. doi: 10.1056/NEJMoa2035716. Epub 2021 Feb 13

Methods: In this phase 3 trial, we randomly assigned (in a 1:1:1 ratio) patients with advanced renal cell carcinoma and no previous systemic therapy to receive lenvatinib (20 mg orally once daily) plus pembrolizumab (200 mg intravenously once every 3 weeks), lenvatinib (18 mg orally once daily) plus everolimus (5 mg orally once daily), or sunitinib (50 mg orally once daily, alternating 4 weeks receiving treatment and 2 weeks without treatment). The primary end point was progression-free survival, as assessed by an independent review committee in accordance with Response Evaluation Criteria in Solid Tumors, version 1.1. Overall survival and safety were also evaluated.

Results: A total of 1069 patients were randomly assigned to receive lenvatinib plus pembrolizumab (355 patients), lenvatinib plus everolimus (357), or sunitinib (357). Progression-free survival was longer with lenvatinib plus pembrolizumab than with sunitinib (median, 23.9 vs. 9.2 months; hazard ratio for disease progression or death, 0.39; 95% confidence interval [CI], 0.32 to 0.49; P<0.001) and was longer with lenvatinib plus everolimus than with sunitinib (median, 14.7 vs. 9.2 months; hazard ratio, 0.65; 95% CI, 0.53 to 0.80; P<0.001). Overall survival was longer with lenvatinib plus pembrolizumab than with sunitinib (hazard ratio for death, 0.66; 95% CI, 0.49 to 0.88; P=0.005) but was not longer with lenvatinib plus everolimus

than with sunitinib (hazard ratio, 1.15; 95% CI, 0.88 to 1.50; P=0.30). Grade 3 or higher adverse events emerged or worsened during treatment in 82.4% of the patients who received lenvatinib plus pembrolizumab, 83.1% of those who received lenvatinib plus everolimus, and 71.8% of those who received sunitinib. Grade 3 or higher adverse events occurring in at least 10% of the patients in any group included hypertension, diarrhea, and elevated lipase levels.

Conclusions: Lenvatinib plus pembrolizumab was associated with significantly longer progression-free survival and overall survival than sunitinib. (Funded by Eisai and Merck Sharp and Dohme; CLEAR ClinicalTrials.gov number, NCT02811861.).

Summary from the Kidney Cancer Association's Inaugural Think Thank: Coalition for a Cure. Rini B, et al. Clin Genitourin Cancer. 2021 Apr;19(2):167-175. doi: 10.1016/j.clgc.2020.10.005. Epub 2020 Nov 14.

Abstract: Close to 74,000 cases of renal cell carcinoma (RCC) are diagnosed each year in the United States. The past 2 decades have shown great developments in surgical techniques, targeted therapy and immunotherapy agents, and longer complete response rates. However, without a global cure, there is still room for further advancement in improving patient care in this space. To address some of the gaps restricting this progress, the Kidney Cancer Association brought together a group of 27 specialists across the areas of clinical care, research, industry, and advocacy at the inaugural "Think Tank: Coalition for a Cure" session. Topics addressed included screening, imaging, rarer RCC subtypes, combination drug therapy options, and patient response. This commentary summarizes the discussion of these topics and their respective clinical challenges, along with a proposal of projects for collaboration in overcoming those needs and making a greater impact on care for patients with RCC moving forward.

TiNivo: safety and efficacy of tivozanib-nivolumab combination therapy in patients with metastatic renal cell carcinoma. Albiges L, et al. Ann Oncol. 2021 Jan;32(1):97-102. doi: 10.1016/j.annonc.2020.09.021. Epub 2020 Sep 30. PMID: 33271289 DOI: 10.1016/j.annonc.2020.11.016

Background: Treatment with tivozanib, a highly selective and potent vascular endothelial growth factor receptor tyrosine kinase inhibitor, has demonstrated single-agent efficacy in advanced renal cell carcinoma (RCC) along with minimal off-target toxicities and a favorable adverse event (AE) profile. We report final results from TiNivo, a phase Ib/II study of tivozanib combined with nivolumab.

Patients and methods: In phase Ib, patients with metastatic RCC received tivozanib 1.0 mg once daily (QD) for 21 days followed by 7 days off treatment (n = 3) or tivozanib 1.5 mg QD (n = 3) plus nivolumab 240 mg every 2 weeks. The maximum tolerated dose was determined to be tivozanib 1.5 mg, and 22 additional patients were enrolled at the maximum tolerated dose for phase II. Primary end points included safety and tolerability, with secondary end points of objective response rate, disease control rate, and progression-free survival.

Results: In total, 25 patients were treated with tivozanib 1.5 mg QD [12 (48%) treatment-naïve; 13 (52%) previously treated]. Treatment-related grade 3/4 AEs were reported in 20 patients (80%); 4 patients (17%) experienced AEs that led to dose reduction, and 8 (32%) discontinued due to AEs. The objective response rate was 56% (including one complete response) and disease control rate was 96%, with a median time to best response of 7.9 weeks. Twenty patients (80%) had tumor shrinkage. With a median follow-up of 19.0 months (range, 12.6-22.8), median progression-free survival was 18.9 months (95% confidence interval 16.4-not reached) in all patients and was similar in treatment-naïve and previously treated patients.

Conclusions: Tivozanib plus nivolumab combination therapy showed a generally tolerable AE profile and promising antitumor efficacy. These results support further development of tivozanib combined with nivolumab as a treatment option in patients with treatment-naïve or previously treated metastatic RCC. Clinical trial number: NCT03136627.

Recent eUpdate to the ESMO Clinical Practice Guidelines on renal cell carcinoma on cabozantinib and nivolumab for first-line clear cell renal cancer: Renal cell carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Powles T; ESMO Guidelines Committee. Ann Oncol. 2021 Mar;32(3):422-423. doi: 10.1016/j.annonc.2020.11.016. Epub 2020 Nov 30. PMID: 33271289

No abstract available

Clear cell renal cell carcinoma ontogeny and mechanisms of lethality. Jonasch E, Walker CL, Rathmell WK. Nat Rev Nephrol. 2021 Apr;17(4):245-261. doi: 10.1038/s41581-020-00359-2. Epub 2020 Nov 3. PMID: 33144689

The molecular features that define clear cell renal cell carcinoma (ccRCC) initiation and progression are being increasingly defined. The TRACERx Renal studies and others that have described the interaction between tumour genomics and remodelling of the tumour microenvironment provide important new insights into the molecular drivers underlying ccRCC ontogeny and progression. Our understanding of common genomic and chromosomal copy number abnormalities in ccRCC, including chromosome 3p loss, provides a mechanistic framework with which to organize these abnormalities into those that drive tumour initiation events, those that drive tumour progression and those that confer lethality. Truncal mutations in ccRCC, including those in VHL, SET2, PBRM1 and BAP1, may engender genomic instability and promote defects in DNA repair pathways. The molecular features that arise from these defects enable categorization of ccRCC into clinically and therapeutically relevant subtypes. Consideration of the interaction of these subtypes with the tumour microenvironment reveals that specific mutations seem to modulate immune cell populations in ccRCC tumours. These findings present opportunities for disease prevention, early detection, prognostication and treatment.

scRNA-seq Analyses Offer Clues to Why Some types of RCC Respond to Immunotherapy While Others Do Not

A new study using single cell RNA sequencing (scRNA-seq) provide insights into the putative cell of origin for RCC subtypes and highlight the important role of the tumor microenvironment in influencing ccRCC biology. The analysis also revealed pathways and interactions within the tumor microenvironment that predicted whether the tumor would respond to immunotherapy.

The study from the University of Michigan Rogel Cancer Center involved an analysis of the RNA of individual renal cells within multiple benign and cancerous kidney tumor. "Single cell RNA sequencing was key to allowing us to monitor gene expression patterns in each individual cell, revealing the mechanisms at play within the tumor microenvironment that can predict overall survival," says study author Arul Chinnaiyan, M.D., Ph.D., director of the Michigan Center for Translational Pathology and S.P. Hicks Professor of Pathology at Michigan Medicine.

The researchers discovered an active role for tumor epithelia in promoting immune cell infiltration, potentially explaining why ccRCC responds to immune checkpoint inhibitors, despite having a low neoantigen burden. "A dichotomy in tumor microenvironment phenotype exists, whereby patients with higher endothelial cell content in primary disease tend to have better overall survival, but metastatic ccRCC patients with high endothelial cell content respond poorly to immunotherapy," the authors write. The authors conclude that the lack of immunotherapy response and endothelial cell fraction has important clinical implications.

Reference: Zhang Y et al.a "Single-cell analyses of renal cell cancers reveal insights into tumor microenvironment, cell of origin, and therapy response," PNAS June 15, 2021 118 (24) e2103240118; PNAS. DOI: 10.1073/pnas.2103240118.

Synergistic Effect of Cancer Vaccine on IO Drugs

A new cancer vaccine which incorporates a new immunostimulant could boost the positive effects of existing immunotherapy drugs, improving the success rate of treatments from 20% to 75% of cases, according to a new study by immunologists from the University of Konstanz. The findings appeared in Nature Communications on May 18, 2021. The preclinical study further demonstrated that combining the vaccine with an immune checkpoint inhibitor can vastly improve the proportion of individuals who respond to treatments, eliminating tumors in 75% of cases in mice. The results suggest that this new approach may be a potent anti-cancer immunotherapy to be tested in future clinical trials.

Although immunotherapy offers major breakthroughs in the therapeutic landscape of many cancers, not all cancer patients respond equally to immunotherapy possibly due to the body's natural down regulation of its immune response. To address such roadblock, a team led by Professor Marcus Groettrup at the University of Konstanz, has developed a microparticle-based cancer vaccine, which uses the immunostimulant Riboxxim that has the approval for application in humans. This vaccine particles measuring 1 micrometre that included a tumor protein and Riboxxim can effectively launches the body's T-cell response that is necessary for immune checkpoint blockade drugs to be effective.

"We were able to formulate a clinically applicable cancer immunotherapy which acts complementary to commonly used immunotherapy," says Dennis Horvath, who is joint-first author on the study together with Julia Koerner at the University of Konstanz (CASCB).

Based on the study's findings, the researchers suggest that these promising pre-clinical results should be transitioned into clinical application. "This might have a very beneficial impact on immunotherapy in certain types of cancer," says Groettrup. This synergistic effect of cancer vaccine is currently being tested in a phase 1 clinical trial in humans.

Reference: Koerner, J., et al. (2021) PLGA-particle vaccine carrying TLR3/RIG-I ligand Riboxxim synergizes with immune checkpoint blockade for effective anti-cancer immunotherapy. Nature Communications. doi.org/10.1038/s41467-021-23244-3.

New Study Finds Routing Brain Imaging Should Be Considered for Patients With Metastatic RCC

Researchers found Occult brain metastases occur in a clinically significant percentage (4.3%) of patients with newly diagnosed advanced metastatic renal cell carcinoma (RCC) suggesting that baseline brain imaging should be considered in most patients with RCC according to a study published in the Journal of the National Comprehensive Cancer Network. Ritesh R. Kotecha, MD, from the Memorial Sloan Kettering Cancer Center in New York City, and colleagues retrospectively reviewed data from 1,689 patients with metastatic RCC who had been considered for clinical trial participation at two institutions between 2001 and 2019 with a median 14.1-month follow-up and had undergone brain imaging in this context without clinical suspicion for brain involvement.

A majority of the 72 patients in this cohort (86%) had 2 or more extracranial sites of disease, including lung metastases (92%). International Metastatic RCC Database Consortium (IMDC) risk status was favorable for 26%, intermediate for 61%, and poor for 13% of these patients. In more than one-third of patients (38.5%), central nervous system involvement was multifocal, and the largest brain metastasis was >1 cm in diameter in 40% of the cohort. In almost all patients (93%), localized brain-directed therapy (predominantly radiotherapy) was pursued. Median overall survival was 10.3 months, while the 1-year overall survival probability was 48%. There was no association noted between IMDC risk and number or size of lesions and survival.

"Brain imaging is routinely obtained for patients with kidney cancer and symptoms that suggest central nervous system (CNS) metastases, but none of the patients with brain metastases included here were symptomatic," added senior researcher Martin H. Voss, MD, from Memorial Sloan Kettering Cancer Center. "In current practice, the chest, abdomen, and pelvis are routinely imaged from the time that metastatic disease is first detected, yet many oncologists do not image the brain."

"The retrospective study by Kotecha et al demonstrates that incidental brain metastases occur in a clinically significant percentage of patients with newly diagnosed metastatic RCC and study underscores the utility for magnetic resonance imaging of all patients with metastatic RCC both at initial diagnosis and at regular intervals to detect occult brain metastases, since specific treatment strategies are required for this patient population."" commented Eric Jonasch, MD, Professor of Genitourinary Medical Oncology at The University of Texas MD Anderson Cancer Center, who was not involved in this research.

References: 1. Kotecha RR, Flippot R, Nortman T, et al. Prognosis of incidental brain metastases in patients with advanced renal cell carcinoma. J Natl Compr Canc Netw. 2021;19(4):432-438. doi: 10.6004/jnccn.2020.7634

2. JNCCN Study: Important Potential Role for Routine Brain Imaging in Advanced Kidney Cancer. Published online April 13, 2021. Accessed April 19, 2021. https://prn.to/3xgXBWL.

KidneyCancerAssociation®

FREE CME CREDITS

Free Certified Medical Education (CME) sessions for healthcare professionals interested in advancing their clinical knowledge in kidney cancer.

Learn more at **kidneycancer.org/cme-programming**

Offer resources to patients and families:

JUST DIAGNOSED TOOLKIT

Recently diagnosed? Our toolkit will walk you through a new kidney cancer diagnosis step-by-step.

Access the toolkit online at **kidneycancer.org/just-diagnosed**

PATIENT NAVIGATOR PROGRAM

Our Patient Liaison can help locate financial assistance, local support services, or a kidney cancer specialist.

Contact the Patient Liaison and/or request a printed toolkit:

1-800-544-3KCA | patient@kidneycancer.org

KCJ Editorial Advisory

KCJ Author Guidelines

Scope of Manuscripts

Kidney Cancer Journal considers the following types of manuscripts for publication:

- Reviews that summarize and synthesize peer-reviewed literature to date on relevant topics in a scholarly fashion and format.
- Original contributions based on original, basic, clinical, translational, epidemiological, or prevention studies relating to kidney cancer that are well documented, novel, and significant.
- Letters to the Editor on timely and relevant subjects pertaining to the diagnosis and treatment of renal cell carcinoma.
- Clinical case studies.

Manuscript Submission

Authors are required to submit their manuscripts in an electronic format, preferably by email to the Editor-in-Chief, Robert A. Figlin, MD, at robert.figlin@cshs.org. Please provide in a word processing program. Images should be submitted electronically as well. All material reproduced from previously published, copyrighted material should contain a full credit line acknowledging the original source. The author is responsible for obtaining this permission.

Contact information

List all authors, including mailing address, titles and affiliations, phone, fax, and email. Please note corresponding author.

Peer Review and Editing

Manuscripts will be peer reviewed. Accepted manuscripts will be edited for clarity, spelling, punctuation, grammar, and consistency with American Medical Association (AMA) style. Authors whose manuscripts are not initially accepted may have the opportunity to revise the manuscript based on recommendations from peer reviewers and at the discretion of the Editor-in-Chief.

Conflict of Interest

Kidney Cancer Journal policy requires that authors reveal to the Editor-in-Chief any relationships that they believe could be construed as resulting in an actual, potential, or apparent conflict of interest with regard to the manuscript submitted for review. Authors must disclose this information in the covering letter accompanying their submission.

Manuscript Preparation

Length: Full-length manuscripts should not exceed 4000 words, including references. Please limit the reference list to 50 citations. Manuscripts should be accompanied by figures and/or tables. Generally 4-5 figures and 2-3 tables are preferred for each manuscript. Please include a brief description to accompany these items, as well as a legend for all abbreviations. Manuscripts should not contain an abstract but an introduction is recommended.
Spacing: One space after periods. Manuscripts should be double spaced.

References

All submissions should have references that are referred to in the text by superscripted numbers and that conform to AMA style. Example: Lewczuk J, Piszko P, Jagas J, et al. Prognostic factors in medically treated patients with chronic pulmonary embolism. Chest. 2001;119:818-823.

KCJ Open Access information

Publication ethics

As an official publication of the Kidney Cancer Association, Kidney Cancer Journal (KCJ) is committed to maintaining the highest standards of publication ethics and abides by Code of Conduct of Committee on Publication Ethics (COPE), and aims to adhere to its Best Practice Guidelines. Please refer to COPE flowcharts for further guidance. Manuscript authors, editors, and reviewers are expected to be aware of, and comply with, the best practices in publication ethics. Authors are expected to have knowledge of best practice in publication ethics in regard to, but not limited to, authorship, dual submission, plagiarism, manipulation of data/figures, competing interests and compliance with policies on research ethics.

Policy on use of human subjects

The clinical research studies involving the use of human subjects should inform that study has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The manuscript should be in line with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals and aim for the inclusion of representative human populations (sex, age and ethnicity) as per those recommendations. Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed. Patients' and volunteers' names, initials, and hospital numbers should not be used.

Policy on use of animals

Studies involving experiments with animals must state that their care was in accordance with institution guidelines. All animal experiments should comply with the appropriate standard guidelines/act for the care and use of Laboratory animals and the authors should clearly indicate in the manuscript that such guidelines have been followed. Studies involving experiments with animals must state that their care was in accordance with institution guidelines. Authors must state in their manuscript how the identity of the cell line was confirmed.


LENVIMA®
(lenvatinib) capsules | 10 mg and 4 mg
Visit www.LENVIMA.com/hcp
to learn more
Eisai
MERCK
LENVIMA® is a registered trademark used by Eisai Inc. under license from Eisai R&D Management Co., Ltd.
© 2019 Eisai Inc. All rights reserved. Printed in USA/January 2019 LENV-US2729