Inhibiting the Systemic Autophagic Syndrome - A Phase II Study of Hydroxychloroquine and Aldesleukin in Renal Cell Carcinoma Patients (RCC).
A Cytokine Working Group (CWG) Study

Prometheus protocol #: IIT11PLK01

Principal Investigator: Leonard J Appleman, MD, PhD
UPMC Cancer Pavilion
5150 Centre Avenue Pittsburgh, PA 15232
Phone: 412-648-6538

Co-Investigators	**Location**
Joseph Clark, MD	Loyola University
Brendan Curti, M.D.	Earle A. Chiles Research Institute
Marc S. Ernstoff, M.D.	Dartmouth-Hitchcock Medical Center
John Kirkwood, MD	University of Pittsburgh
Theodore Logan, M.D.	Indiana University
Michael Lotze, MD	University of Pittsburgh (Local Co-Investigator)
Jodi Maranchie, MD	University of Pittsburgh (Local Co-Investigator)
David McDermott, MD	Beth Israel Deaconess Medical Center
Paul Monk, MD	The Ohio State University
Stergios Moschos, MD	University of North Carolina
Andrea Facciabene, PhD	University of Pennsylvania

Pathology Core Director
Rajiv Dhir, M.D. UPMC Hillman Cancer Center

Immunologic Monitoring and Cellular Products Laboratories (IMCPL)
Theresa Whiteside, PhD

Statistician
Daniel P. Normolle, PhD UPMC Hillman Cancer Center &
Biostatistics Dept, University of Pittsburgh

Rheumatology
Larry Moreland, MD University of Pittsburgh

Program Manager for GU Clinical Research
Clare Grzejka, RN, BSN University of Pittsburgh

Consultant
Kim Margolin, MD University of Washington, CITN, CWG

Prometheus/Clinigen Contact
Nancy Gregory
Phone: 832-326-3256

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Summary and Schema

Treatment Arm: One course of treatment (84 days) will consist of high dose (600,000 IU/kg) bolus IL-2 administered intravenously every 8 hours beginning on day 1 and day 15 (maximum of 14 doses per cycle of administration); and hydroxychloroquine (HCQ) orally started two weeks prior to IL-2 infusions (+/- 1 day) [to allow for weekends/holidays etc] and continued while able to take oral medication throughout all courses. For patients with evidence of stable disease or response up to three courses can be administered as shown below. Patients will be administered 600mg/d of HCQ at all sites in a total group of 21.

Course 1

Drug	**Days**: -14	1	15	29	43	57	71	84
Hydroxychloroquine	X	----------	----------	----------	----------	----------	----------	X
IL-2		X	X					
Biomarker	X	X	X					

Course 2

Drug	**Days**: 85	99	113	127	141	155	168
Hydroxychloroquine	X	----------	----------	----------	----------	----------	X
IL-2	X	X					
Biomarker	X						

Course 3

Drug	**Days**: 169	183	197	211	225	239	252
Hydroxychloroquine	X	----------	----------	----------	----------	----------	X
IL-2	X	X					
Biomarker	X						

Agents

- Hydroxychloroquine oral at 600 mg/d throughout the treatment. Continuous oral administration will be initiated prior to the first dose (day -14), given a minimum of 14 days (maximum of 21 days) prior to initiation of the first dose of IL-2 and then daily or twice a day throughout all three treatment courses.

- **Aldesleukin** (IL-2) 600,000 IU/kg IV bolus q 8 hrs beginning on day 1 and day 15 (maximum 14 doses per cycle).

Response Assessment

- Patients will undergo CT scans at week 8 (+/- 1 week) and week 12 (+/- 1 week), then after both subsequent courses (every 10-12 weeks). After the final course (week 36), patients will undergo CT scan evaluation every 12 weeks (+/- 2 weeks) for up to one year or until tumor progression (whichever comes first). If additional scans are performed for clinical reasons, the study schedule may be altered to perform the requisite scans at a more acceptable time point. The change in timing of study scans must be approved by the local PI and documented. For patients who have not shown progression at the end of one year, response will be captured during follow-up forsurvival, which will be done on all patients via telephone contact and medical record review.

- Hydroxychloroquine will continue daily until documented disease progression or 12 weeks following documented complete response.

- Patients with evidence of tumor shrinkage may receive up to 3 courses of IL-2 therapy. Course 2 will start on or within 2 weeks following day 85 and follow the same schedule as in Course 1. Course 3 can start within 2 weeks of completion of course 2. Patients not receiving IL-2 during course 2 who have some evidence of tumor shrinkage by week 24 can receive a 2nd course of IL-2 beginning within 2 weeks of week 25.

- Patients with no evidence of progression by RECIST 1.1 criteria may receive continued treatment with hydroxychloroquine through one year of initiation of HCQ treatment (Day -14) or until disease progression.

- Patients with progressive disease by RECIST 1.1 criteria will be taken off-study.

- Both responding and non-responding patients will be followed until death or final closure of the study (6.4.2). Attention will be paid to the time of disease progression and time of initiation of alternate therapies.

1.0 OBJECTIVES

We hypothesize that IL-2 administration enhances systemic autophagy, limiting the effectiveness of immune effectors and that coadministration of the autophagy inhibitor hydroxychloroquine will enhance response, diminish toxicity other than possibly GI toxicity, and increase the ability to administer IL-2.

1.1 Primary:

1. Estimate the proportion of patients with metastatic RCC treated with IL-2 combined with hydroxychloroquine at 600 mg/d who experience a clinical complete response.

1.2 Secondary:

2. Compare the distribution of clinical responses, overall survival and progression-free survival of patients with metastatic RCC treated with IL-2 combined with hydroxychloroquine to *the published response of patients treated with high dose IL-2 alone (Proclaim database and publication)*.

3. Characterize the safety and toxicity of IL-2 combined with hydroxychloroquine:

- **a.** Number of doses of IL-2 administered during the first course of therapy
- **b.** Toxicity after the scheduled 9th dose of IL-2
- **c.** Frequency of grade III and IV or unexpected or rare toxicities [Section 9.5]

4. Determine the relationship between laboratory parameters (Sections 7.2-7.5) evaluated at baseline and during treatment and toxicity, clinical response, and survival.

5. Evaluate the utility of known prognostic criteria for RCC patients on clinical outcome. (1). Pretreatment features associated with a shorter survival are low Karnofsky performance status; (<80%), high serum lactate dehydrogenase (> 1.5 times upper limit of normal), low hemoglobin (< lower limit of normal), high "corrected" serum calcium (> 10 mg/dL), and absence of prior nephrectomy (see 13.2.4).

2.0 BACKGROUND AND RATIONALE

2.1 Treatment of RCC- Background

In 2010, it is estimated that there will be 35,710 new cases of kidney cancer and approximately 12,480 kidney cancer-specific deaths in the United States (2). One-third of patients have metastatic disease at the time of diagnosis. Two recent randomized studies in metastatic RCC patients have shown survival benefit of nephrectomy and cytokine therapy over cytokine therapy alone (3, 4). For this reason, and for symptom control, many patients with metastatic disease at presentation undergo nephrectomy as a component of their standard care. The prognosis for recurrent or metastatic renal cell carcinoma is poor. Median survival was 10-13 months prior to the development of agents targeting the VEGF and mTOR pathways, and is now approximately 2 years in good- and intermediate-prognosis patients (5) and 5-year survival is less than 5%. These figures underscore the need for effective systemic therapy in this disease.

Medical treatment for RCC has primarily focused on biological therapies designed to mobilize immune effector cells that recognize and destroy cancer cells (3-9). Several randomized studies have suggested that interferon produces modest antitumor benefit in patients with advanced renal cancer, while IL-2 has received FDA approval due to its ability to produce durable responses, albeit in only a minority of patients. Tumor infiltrating lymphocytes are often NK cells, which, when present in high numbers, are associated with improved prognosis (see below).

2.2 High-dose IL-2 Therapy for Patients with RCC

Investigations of IL-2 (Aldesleukin, Chiron) in patients with RCC were initiated by our group [Surgery Branch] at the NCI in the mid-1980s,and continued there and within the Cytokine Working Group. Initial studies evaluated high-dose bolus IL-2 therapy in combination with the infusion of autologous lymphocytes activated *ex vivo* in IL-2 (lymphokine-activated killer or LAK cells) (10, 11). While dramatic and durable responses were reported in some patients, subsequent studies found the efficacy of high-dose bolus IL-2 alone to be equivalent to that of the combination of IL-2 and LAK cells, prompting the abandonment of this more complex cellular therapy component.

In 1992, high-dose bolus IL-2 was approved by the FDA for the treatment of patients with metastatic renal cell cancer based on data presented on 255 patients entered onto 7 phase II clinical trials (8, 12). In these studies, 600,000-720,000 IU/kg of recombinant human IL-2 was administered by 15-minute infusion every 8 hours x 14 doses, thereby constituting a course of therapy. Patients received a course of therapy, consisting of two 5-day treatment cycles separated by 5-9 days of rest (maximum of 28 doses) (cycle 1A/1B). Courses were repeated every 8-12 weeks in stable or responding patients. Although 35% of patients received 720,000 IU/kg of IL-2 per dose and the remainder received 600,000 IU/kg per dose, the median cumulative amount of IL-2 administered was the same in both groups, since patients receiving 720,000 IU/kg per dose tolerated fewer IL-2 doses. Ninety-six percent of these patients had an ECOG performance status of 0 or 1, 85% had undergone a nephrectomy prior to starting IL-2 therapy, none had received prior immunotherapy, and the median time from diagnosis to treatment was 8.5 months.

Objective responses were seen in 37 of the 255 patients (RR 15%). There were 17 (7%) complete responses (CRs) and 20 (8%) partial responses (PRs). Fourteen of the responding patients (38%) began therapy with tumor burdens >50 cm^2 on pretreatment scans and 60% of PRs had >90% regression of all measurable disease. The median duration of response was 54 months for all responders, 20 months for PRs and has not yet been reached for CRs. The median survival was 16 months for all 255 patients.

Follow-up data on these patients has now been accumulated through June 2010, with a median follow-up of over 10 years. Although some late relapses are still being observed, the response duration curve appears to have leveled off after the 30 month time point and 60% of complete responders remain in remission. In addition, 4 PRs who underwent surgical resection of residual disease while still in response remain alive and disease-free at a minimum of 65+ months. Therefore, many CRs remaining free from progression for more than thirty months and those PRs resected to NED after a response to high-dose IL-2 are unlikely to progress and may actually be cured. Thus, although the response rate to high-dose bolus IL-2 is modest, it can produce meaningful benefit in a small proportion of patients. Means to enhance this response are clearly in order.

The High Dose Aldesleukin "Select" trial was conducted to prospectively assess the ability of histologic, immunohistohemical and other pathologic tumor features to predict overall response rate and other outcomes (McDermott et al. 2011). This study enrolled 123 patients between November 2006 and July 2009, at many of the same Cytokine Working Group Institutions participating in the present study. Patients received high dose aldesleukin at a standard dose and schedule, and with standard recommendations for treatment modification for toxicity (Schwartzentruber 2001). The High Dose Aldesleukin ="Select" study therefore represents a contemporaneous population of patients with similar baseline characteristics and aldesleukin treatment with which to compare to the current study with regard to outcomes and toxicity."

In that trial, overall response rate was 25% by independent review (95% CI 17.5-33.7) and 28% by investigator review (as to be performed in this study) (95% CI 20.5-37.3%). The distribution of MSKCC 2002 risk was 19/70/11% (Good/Int/Poor). Median PFS was 4.3 months (95% CI 2.5-4.7 mo) (comparable to prior studies where about half the patients progressed on the first scan). As seen previously, there was a "tail" of durable progression-free survival (PFS rate at 3 years was 11%). Median overall survival was 42.8 months (95% CI 35.6-51.9 months). Median duration of response was 20.6 months (CI 6.9-42.7 mo)

2.3 Randomized Trials with IL-2

Other regimens involving lower doses of IL-2 with and without interferon (IFN) have been reported to produce similar response rates to those observed with high dose (HD) IL-2, but these results have not been reproducible and the quality of responses has been less impressive (9,13,14). Before accepting lower dose regimens as equivalent to HD IL-2, it was imperative to establish that the quality of tumor responses with lower dose regimens was not inferior. Three large-scale randomized trials have now been completed that provide clinicians with useful insights into the relative merits of these various regimens and thus the optimal management of patients with metastatic renal cell carcinoma.

Investigators in France conducted a large-scale, phase III randomized trial comparing intermediate-dose IL-2 administered by continuous intravenous infusion plus subcutaneous IFNα with either IL-2 or IFNα administered alone (9). Four hundred twenty-five patients were enrolled. The three treatment groups were well balanced for age and gender as well as known predictors of response and survival. The response rate and 1-year event-free survival were significantly greater for the combined IL-2 and IFNα arm than for either of the single-agent arms, although there was no significant difference in overall survival among the three groups. Of note, responses were seen in only 6.5% and 7.5% of patients receiving IL-2 or IFNα alone, respectively, with only 2.9% and 6.1% of these patients still responding at the week 25 evaluation. Although more anti-tumor activity was seen with the combination arm, this was largely due to the rather limited activity of the single-agent regimens. How an intermediate-dose combination of IL-2 and IFNα would compare with high-dose IL-2 alone remained to be established.

The NCI Surgery Branch investigators performed a randomized trial comparing standard HD IL-2 and the intermediate dose (low dose – LD) intravenous bolus IL-2 regimen (LD IV IL-2) (15). After randomizing 117 patients, a third arm was added involving subcutaneous IL-2 administered in a fashion similar to that previously described by Sleijfer *et al*. Results were analyzed and reported according to groups that were concurrently randomized. Among the 306 patients concurrently assigned to either HD or LD IV IL-2, the response rate was significantly higher with high-dose therapy (21 versus 13 percent), with a trend towards more durable responses. Response durability and survival in complete responders was superior in patients who received the HD intravenous IL-2 compared to those who received the LD IV IL-2. There were no differences in overall survival. Although toxicities were also significantly greater in the high dose group (particularly hypotension), there were no deaths attributable to IL-2 in either arm and patient assessments of quality of life were found to be roughly equivalent. Among the patients concurrently assigned to either subcutaneous IL-2 or HD intravenous bolus IL-2, a higher response rate was seen with HD IL-2 (21 versus 10 percent) but the difference was of borderline statistical significance. Once again there were no differences in overall survival.

In an effort to determine the value of outpatient subcutaneous IL-2 and IFNα relative to high dose IL-2, the Cytokine Working Group performed a phase III trial in which patients were randomized to receive either outpatient IL-2 (5 MIU/m^2 subcutaneously every 8 hours x 3 doses on day 1 then daily 5 days/week for 4 weeks) and IFNα (5 MIU/m^2 subcutaneously thrice weekly for 4 weeks) every 6 weeks or standard high dose inpatient IL-2 (600,000 IU/kg/dose every 8 hours intravenously, days 1-5 and 15-19 [max 28 doses]) every 12 weeks (16). One hundred and ninety three patients were enrolled and 192 were evaluable. Patients were stratified for bone or liver metastases, primary in place and performance status 0/1 and the treatment arms were evenly balanced for these characteristics as well as other factors. 45% of patients had bone or liver metastases, 31% had primary in place and 60% were PS 0. Toxicities seen were typical for these regimens, including one treatment related death from progressive disease and ARDS on IL-2 and IFN arm and one death from capillary leak syndrome on high dose IL-2.

The response rate for high-dose IL-2 was 23% (22/95) versus 10% (9/91) for IL-2/IFN (p=0.018). Eight patients achieved a complete response on high dose IL-2 versus only 3 on low dose IL-2/IFN. The median response durations were 24 months for high dose IL-2 and 15 months for IL-2/IFN (p=0.18). Median overall survivals were 17 and 13 months (p=0.21), favoring high dose IL-2. Ten patients on high dose IL-2 were progression free at 3 years versus 3 on IL-2/IFN (p=0.08). Durable CRs favored HD IL-2 (7 vs. 0). Median progression free survival was 3 months for each

treatment arm. Of note, responses to high dose IL-2 were seen with equal frequency across the stratification criteria, while low dose IL-2/IFN appeared to produce fewer responses in patients with liver and/or bone metastases and in those who had not undergone prior nephrectomy to remove the primary tumor. For patients with bone or liver metastases (p=0.001) or primary in place (p=0.04) survival was superior with high dose IL-2 compared to IL-2/IFN, while no significant survival differences between the two treatments were noted for patients who had undergone prior nephrectomy or were without bone or liver metastases. Moreover, patients who had undergone a recent nephrectomy (debulking nephrectomy in the setting of stage IV disease) appeared to fare as well with IL-2/IFN as with HD IL-2.

Taken together these studies suggest that HD bolus IL-2 is superior in terms of response rate and response quality to regimens involving either low dose IL-2 and IFN or intermediate or low dose IL-2 or subcutaneous IFN. Furthermore, the benefit of high dose IL-2 relative to the lower dose regimens may be most pronounced in patients with unresected primary tumors or bone and/or liver metastases. This suggests that high dose IL-2 is required to produce responses in typically refractory sites and to eliminate the last tumor cell. This data suggests that high dose IL-2 should remain the preferred therapy for appropriately selected patients with access to such therapy. However, given the toxicity and limited efficacy of high dose IL-2 therapy, additional efforts should be directed at better defining the patient population for whom this therapy is appropriate and finding means to ameliorate toxicity and increase efficacy.

2.4 Predictors of Clinical Benefit from Cytokine-Based Therapy

Many groups have attempted to determine reliable predictors of response and survival for patients with metastatic renal cell carcinoma who were receiving immunotherapy. Factors that have been variably associated with response include performance status, number of organs with metastases (one versus two or more), absence of bone metastases, prior nephrectomy, degree of treatment-related thrombocytopenia, absence of prior interferon therapy, thyroid dysfunction, rebound lymphocytosis, erythropoietin production, and post-treatment elevations of blood TNF-α and IL-1 levels (17-20).

Motzer and colleagues have shown in patients receiving IFN that poor survival is associated with low Karnofsky performance status, high serum lactate dehydrogenase, low hemoglobin, high "corrected" serum calcium and time from initial renal cell carcinoma diagnosis to start of therapy of less than one year (20). In a cohort of 453 patients who received IFN as initial therapy, the median survival for the favorable (no risk factors), intermediate (one or two risk factors) and poor (three or more risk factors) risk groups were 30, 14 and 5 months respectively. Negrier and associates also identified independent predictors of rapid disease progression, defined as progression within 10 weeks of initiation of therapy (19, 21). These included greater than one metastatic site, disease-free interval of less than 1 year, and presence of liver metastases or mediastinal nodes, as well as type of immunotherapy used. Patients with liver metastases, more than one site of disease, and disease-free interval of less than 1 year had a lower response rate and a median survival of only 6 months, even while receiving combination IL-2 and interferon α therapy. Figlin and colleagues identified prior nephrectomy and time from nephrectomy to relapse as important predictors of survival in patients receiving IL-2-based therapy (10). In their series, patients who received systemic immunotherapy for metastatic disease more than 6 months after nephrectomy had the best median survival and had a 3-year survival rate of 46%. A recent multivariate analysis by the same group of investigators that was confined to patients who received IL-2 after nephrectomy revealed survival to be inversely

associated with lymph node involvement, constitutional symptoms, sarcomatoid histology, metastases involving sites other than bone or lung or multiple sites, and a TSH level > 2.0 mIU/L (10). They proposed a scoring algorithm based on these features in which survival at one year was predicted to vary from 1- 92%. Recent data from the Cytokine Working Group phase III trial, mentioned above, suggested that disease site factors such as primary in place or hepatic or bone metastases may be more predictive of a poor response to low dose IL-2 and IFN regimens, than to high dose IL-2. Furthermore, this study suggested the greatest benefit from high dose IL-2 relative to lower dose regimens might be seen in patients with primaries in place and/or liver and bone metastases. These data call into question some of the prior studies and suggests that additional predictors of response and survival in patients receiving cytokine-based immunotherapy are necessary.

2.5 Influence of subtype on response to IL-2

Responses to immunotherapy are most frequently seen in patients with renal cell carcinoma of clear cell histology. This observation was recently detailed in a retrospective analysis by Upton et al of pathology specimens obtained from 231 patients who had received IL-2 therapy on Cytokine Working Group clinical trials (22). For patients with tumor specimens available for review, the response rate to IL-2 was 21 percent (30 of 146) for patients with clear cell histology primary tumors, compared to 6 percent for patients with non-clear cell histology (1 responder in 17 patients). Among the patients with clear cell cancer in their kidney specimen, response to IL-2 was also associated with the presence of > 50% alveolar features, no papillary features or granular features in the pathologic material. The response rate was 39 percent (14 of 36) in patients with all these features. In addition, patients with clear cell tumors with < 50% alveolar or granular features but no papillary features in the specimen had a response rate of 19 percent (15 of 77) compared to 3 percent (1 of 33) in the other patients.

From this data, a model was developed that placed patients with clear cell cancers into good, intermediate and poor response categories based on the analysis of their primary tumors.

When this model was then applied to the 68 patients with specimens from other metastatic sites, all 7 tumor responses were seen in the 42 patients with clear cell cancer and intermediate or good prognostic features, thus confirming the validity of the model developed from the primary kidney tumor specimens. In addition, median survivals for all patients with clear cell tumors by risk group were 2.87, 1.36 and 0.87 years, respectively ($p < 0.001$), indicating that the model may have prognostic as well as predictive value.

Table 1. Histologic Predictors of Response to IL-2 Therapy

Category	Pathology Type	N	Response	RR %
Good	Clear Cell Alveolar > 50% No granular No papillary	36	14	39
Intermediate	Clear Cell Alveolar <50% Granular <50% No papillary	77	15	19
Poor	Others	33	1	3

As a result of these data, it has been conjectured that patients with non-clear cell histology and those with clear cell histology but adverse pathologic features (papillary and/or no alveolar features, and/or >50 percent granular features) receive alternative non-immunotherapy-based treatments; however, this supposition requires confirmation using an independent data set. In addition, the encouraging results in the favorable pathology types require prospective confirmation to determine the true response rates in a selected population. The association of response with pathologic features suggests that more intensive evaluation of tumor tissue might yield more robust predictors of response. Given that even in the most favorable prognostic group only 39% of patients responded to IL-2 therapy, additional investigations into tumor-associated predictors of responsiveness to IL-2 are still warranted.

2.6 Molecular Markers of Response to IL-2

Some investigators have begun to examine tumor tissue to identify molecular markers that might predict the outcome of patients with renal cell carcinoma. Recently, carbonic anhydrase IX (CAIX) has been identified as one potential marker (23). CAIX is thought to play a role in cell proliferation in response to hypoxic conditions. CAIX expression is mediated by the HIF-1 alpha transcriptional complex and induced in many tumors types, but is absent in most normal tissue with the exception of epithelial cells of the gastric mucosa. Bui *et al* used a monoclonal antibody designed to detect CAIX expression to perform an immunohistochemical analysis of paraffin embedded renal cell carcinoma specimens (23). They showed that > 90% of renal cell carcinomas express CAIX and that its expression decreases with advancing stage. In their analysis, high CAIX expression in primary tumors was seen in 79% of patients and was associated with improved survival and, possibly, response to IL-2-based therapy. In addition, all long-term responders to IL-2-based treatment had high CAIX expression. In this study, low CAIX expression was associated with a worse outcome for patients with locally advanced renal cell carcinoma and was an independent predictor of outcome in patients with metastatic disease.

Building on this work, Atkins et al performed a nested case-control study within the larger Upton pathology cohort (24). Paraffin-embedded tissue sections of RCC were obtained from patients who had previously been included in the pathology review and were immunostained with the same CAIX antibody used by Bui et al. CAIX expression levels were correlated with type of IL-2 treatment, response to IL-2, pathologic risk categorization and survival. As in the report by Bui *et al*, the percentage of CAIX positive tumor cells was utilized to separate high (> 85%) and low (≤ 85%) expressors. Tissue specimens were obtained from 66 patients. Twenty-seven of the selected patients (41%) achieved a response (10CR/17PR) to high-dose IL-2 (32) or low-dose IL-2-based

(34) regimens with 20 (30%) remaining alive at a median follow-up of 2.6 years. Fifty-eight specimens were assessed as clear cell, with 56, 33 and 4 having alveolar, granular and papillary features, respectively. Twenty-four (36%), 31 (47%) and 11 (17%) were classified into good, intermediate, or high-risk groups according to the pathology model created by Upton *et al* that is described above. Forty-one specimens (62%) had high CAIX expression. Twenty-one of 27 (78%) responding patients had high CAIX expression, compared to 20/39 (51%) of non-responders (OR=3.3, p=0.04). A similar survival benefit was observed (p=0.04). Median survival was 3 and 1 years for high and low CAIX expressers, respectively. Survival greater than 5 years was only seen in the high CAIX expressing group. High CAIX staining was associated with better pathology prognosis and, when stratified by group, the odds ratios of CAIX expression were unequal across groups. The strongest association was in the intermediate pathology group where 9/9 responders had high CAIX expression vs. 11/22 non-responders, suggesting a possible refinement of the Upton pathology model. A resultant group with good pathology or intermediate pathology and high CAIX expression contained 26 of 27 (96%) responders compared to only 18/39 (46%) non-responders (OR = 30; p <0.01). Survival benefit was also seen (p< 0.01). The authors concluded that based on this data, patients with low CAIX staining and intermediate or poor risk pathology classification should be considered for non-IL-2 based treatments.

Table 2. CAIX Staining and Response Rate

Category	N	Response Rate %
Low CAIX staining Intermediate or poor path	22	<5%
Other	44	59%

While this model requires validation, it is possible that IL-2 therapy could ultimately be reserved for patients with favorable or intermediate pathology classification and high CAIX staining who appear to be more likely to benefit.

Additional studies to explain these preliminary observations and correlate results with pathologic features, sites of disease and previously described clinical features are necessary. In addition, gene expression profiling of tumor specimens to identify new proteins or patterns of gene expression that might be associated with IL-2 responsiveness may eventually help narrow the application of IL-2 therapy to those who will benefit the most. The SELECT Cytokine Working Group has recently concluded that combined histologic subtype and CAIX predictors were unable to define a predictor of response (McDerrmott et al, 2017; 150).

2.7 Application of models to high dose IL-2 population

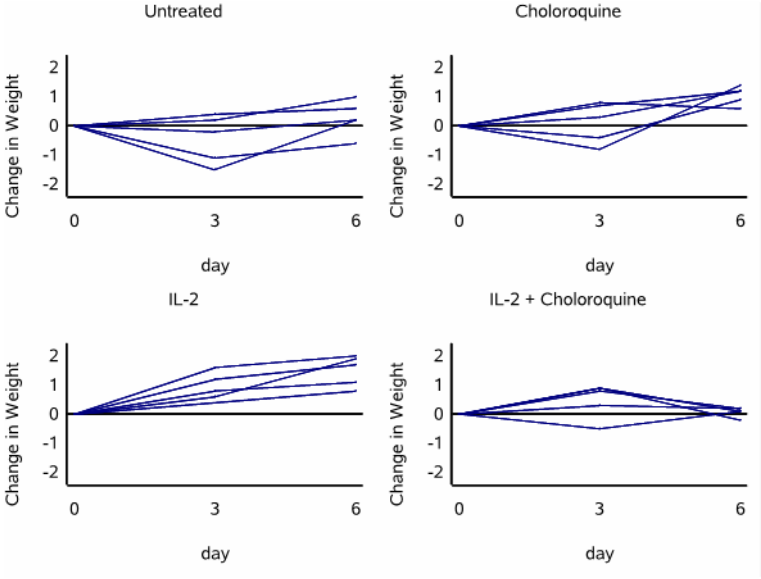
The pathology and combined CAIX plus pathology models were retrospectively applied to the population of patients treated with high dose IL-2 on the Phase III Cytokine Working Group trial. Fifty-five patients who received high dose IL-2 and had their pathology reviewed as part of the IL-2 pathology analysis were classified into either the good or intermediate prognostic category. In this group of patients, the overall response rate was 31% (17 of 55), the CR rate was 11% (6 of 55), the 3 year progression free survival rate was 14.5% (8/55), and the median progression free survival was 4.7 months. In addition, thirty-two patients who received high dose IL-2 and were included in the CAIX staining project had either good pathology or intermediate pathology and high CAIX staining. These 32 patients had an overall response rate of 37.5% (12/32), a complete response rate of 16% (5/32), a 3-year progression free survival rate of 19% (6/32) and a median

PFS of 6.4 months. Considering that the CAIX staining population was enriched for responding patients, these latter numbers probably overestimate the true efficacy of high dose IL-2 in this population. On the other hand, the pathology analysis contains patients with intermediate pathology and low CAIX staining that may depress the overall clinical efficacy. Based on this data one would predict that patients selected for IL-2 therapy according to the combined CAIX and pathology model would exhibit a response rate of approximate 30-35%, a CR rate of 10-15%, a 3-year progression free survival rate of 12-18%, and a median PFS of 4.5-6 months. As both analyses are heavily confounded with the data used to create the models, prospective confirmation is required. Although supported by substantial information, the recent studies from the Cytokine Working Group [David McDermott, ASCO 2010] do not substantiate an improved outcome for patients predicted to have better response based on these criteria although a 25% response rate was confirmed. We have also suggested little value of the administration of antibodies to VEGF with IL-2 treatment (25-28).

2.8 Autophagy serves as a survival pathway for stressed tumor cells enhancing survival following cytotoxic chemotherapy.

Autophagy is an important highly evolutionarily conserved catabolic process by which cells shuttle effete or damaged organelles and proteins to the autophagosome for sequestration and destruction. In addition to its homeostatic role, the autophagic process also allows cells to survive periods of nutrient deprivation and stress. Autophagy is thought to maintain cellular metabolism through increased turnover of cell components during times of nutrient deprivation. A growing body of literature suggests that autophagy is in fact a form of programmed cell survival and is induced to prevent apoptosis or necrosis (29-31). As is the case in normal cells, autophagy is induced in tumor cells during times of metabolic stress. In fact, several studies have demonstrated that markers of increased autophagic flux localize to metabolically stressed areas of tumor (32). In particular, a recent study by Fujii et al. examined 71 resected adenocarcinomas and demonstrated a high correlation between worse prognosis and increased levels of autophagy in the specimens (33). That autophagy can protect tumor cells during times of stress has led to increased interest in blocking this process as a means of anti-tumor therapy. Inhibition of autophagy as a single agent is unlikely to have much clinical impact since only a small fraction of the tumor cells are under metabolic stress at any given time. Therefore, most strategies have focused on the combination of autophagy inhibition in combination with agents that induce cellular stress (34, 35). Most modern neoplastic therapies fall into this category inducing cellular stress either by genotoxic injury, metabolic insult or blockade of growth factor activity. As shown in **Figure 1**, administration of chloroquine, an inhibitor of autophagy at 50mg/kg/d limits the weight gain in mice observed with high dose IL-2 administration [$6x10^5$ IU bid x 5D] concurrent with administration.

Figure 1. Chloroquine limits IL-2 Induced Vascular Leak and Weight Gain. Weight gain observed with IL-2 administration was ameliorated with chloroquine coadministration. Shown in the ordinate/y axis is the change in weight for each animal over the days of treatment. IL-2+CQ versus IL-2, Days 3 and 6; p=0.04

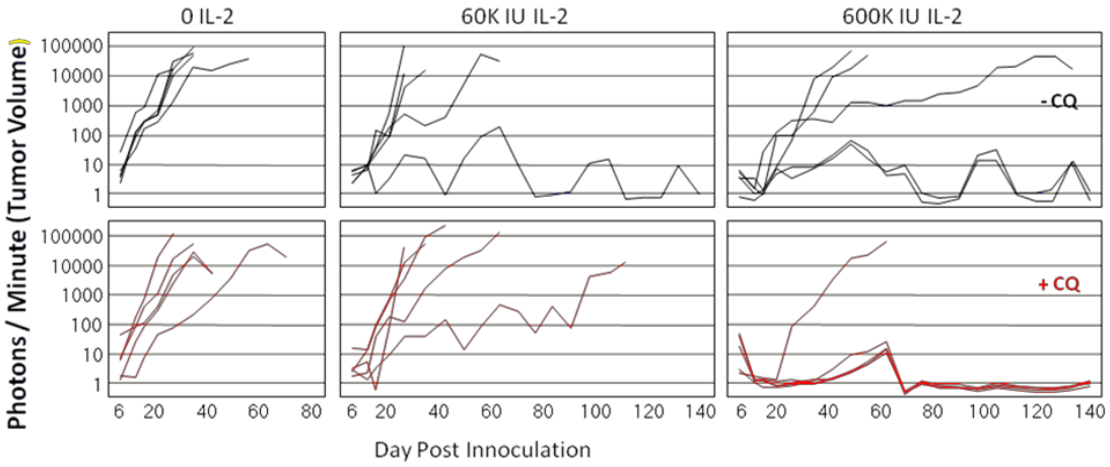


2.9 Chloroquine and its derivatives inhibit autophagy and enhance immunotherapy and chemotherapy effects.

Chloroquine (CQ) is a synthetic 4-aminoquinoline that has been used for 60 years in humans initially for malaria prophylaxis and treatment, and with emergence of resistance, in patients with systemic lupus erythematosus, rheumatoid arthritis, and human immunodeficiency virus (HIV). It is an inexpensive orally available drug with a large therapeutic index. Chloroquine derivatives such as HCQ are still used extensively in patients with rheumatoid arthritis and lupus erythematosus. Chloroquine blocks acidification of the lysosome, thus inhibiting the last step in autophagy. With this last step blocked, a cell reliant on autophagy will increase the generation of autophagosomes and will eventually undergo either apoptotic or non-apoptotic cell death. Evidence in mouse models and human cancer cell lines suggest CQ may have significant anti-tumor activity by inhibiting autophagy induced by cancer therapy (35). Hydroxychloroquine is generally considered the safest disease-modifying agent currently used to treat rheumatic diseases. Up to 10% of patients will experience nausea, headache, or bizarre dreams, but these are infrequently dose-limiting. Retinal toxicity is a rare complication, estimated to occur in <1% of patients treated for years at doses not exceeding 6.5 mg/kg/day lean body weight, or approximately 400 mg/day (36, 37). Given the prolonged half-life of this drug (t1/2=40=45 d), treatment with daily doses of up to 1200 mg daily for a maximum of one month would achieve plasma concentrations approximating steady state with 400 mg daily dosing, and the risk of retinal toxicity for this relatively short-term exposure is thus low. While data is sparse regarding precise risk at higher daily dosing, Shearer and Dubois reported only a single patient with retinopathy in a survey of 94 patients treated with HCQ 800 mg daily for up to 54 months (38). Levy et al. identified 78 patients receiving a daily dosage >7.8 mg/kg, and of the 3 with possible or definite retinal toxicity, HCQ was taken for at least 3.5 years (36). Because retinal toxicity may progress after drug cessation (39), HCQ will be discontinued in any patients reporting any visual complaints. A formal

ophthalmic evaluation plus a 3 month follow-up examination will be obtained only with ocular symptoms.

2.10 Murine Models of IL-2 and Chloroquine [Figure 2]



Kang R, Livesey KM, Zeh HJ, Lotze MT, Tang D. HMGB1: A novel Beclin 1-binding protein active in autophagy. Autophagy. 2010 Nov 16;6(8)

Chavez AR, Buchser W, Basse PH, Liang X, Appleman LJ, Maranchie JK, Zeh H, de Vera ME, Lotze MT. Pharmacologic administration of interleukin-2. Ann N Y Acad Sci. 2009 Dec;1182:14-27.

We have tested IL-2 in combination with chloroquine in mouse models of metastatic colorectal cancer to the liver using luciferase transfected tumor and imaging strategies. When applying high dose IL-2 [$6x10^5$IU bid x 5D] as opposed to low dose regimens [$6x10^4$IU bid x 5D] in the setting of these models, we were able to demonstrate substantial antitumor effects in combination with chloroquine at 50mg/kg/d. Lower doses and dosing frequency were less effective.

2.11 Hydroxychloroquine is more feasible than chloroquine.

Hydroxychloroquine is commonly prescribed for rheumatoid arthritis and lupus at a dose of 400 mg po qd. A pharmacokinetic/pharmacodynamic study of escalating doses of HCQ at 400 mg/800 mg/1200 mg po qd in patients with rheumatoid arthritis followed by maintenance doses of 400 mg po qd found that doses of up to 1200 mg po qd were well tolerated (40). Dose-limiting toxicities (nausea, vomiting and abdominal pain) were observed at 800 and 1200 mg po qd. This toxicity correlated with blood HCQ levels, but not to blood levels of the other active metabolites DHCQ, DCQ, or BDCQ. Improvement of symptoms in rheumatoid arthritis correlates with blood DHCQ levels, suggesting a dose-response relationship. Chloroquine derivatives are metabolized through the p450 enzyme system, and CQ may inhibit the metabolism of CYP2D6- metabolized drugs. A predictable cumulative toxicity associated with CQ is retinopathy, and this is another reason why dose escalation in chronic treatment with CQ could be limited. While a link between HCQ and retinopathy has also been made, it occurs infrequently and only after prolonged exposure. In a study using multifocal electroretinography to detect early pre-clinical retinal changes in long-term HCQ users, 10 out of the 11 patients that developed early pre-clinical changes had been taking HCQ at doses of >400 mg po qd for greater than 5 years (41). No overt retinopathy was noted in the 19 patients followed. This suggests that at a cumulative dose of 730 g. the risk of retinal

changes increase, but techniques such as multifocal electroretinography can detect early changes and prevent overt visual loss.

2.12 Clinical trials examining hydroxychloroquine and chemotherapy for the treatment of human cancers.

The above cited literature, demonstrating the utility of blocking autophagy alone or in combination with cytotoxic chemotherapy *in vitro*, and preclinical models, has led to the initiation of human clinical trials. Currently, there are more than twelve clinical trials examining the combination of hydroxylchloroquine and conventional chemotherapeutic agents. These are summarized briefly on the next page. In dose escalation trials performed at the University of Pennsylvania, doses of 1200mg daily have been achieved with no significant toxicity attributed to the HCQ in various combination including dose intense temazolamide, Velcade, or temsiloimus. In a protocol using temazolamide and radiation therapy in combination with HCQ for patients with Glioblastoma, 800 mg was associated with myelosuppression and the MTD defined as 600 mg/d [Personal Communication, Ravi Amaravadi, MD].

Table 3. Ongoing Clinical Trials of Hydroxychloroquine in Oncology.

Condition	Intervention	Phase	Sponsors Collaborators	ClinicalTrials.gov Identifier	Title
Prostate cancer	Docetaxel hydroxy-chloroquine	II	CINJ, NCI	NCT00786682	A Phase II Study of Docetaxel and Modulation of Autophagy with Hydroxychloroquine for Metastatic Hormone Refractory Prostate Cancer
Prostate cancer	Hydroxy-chloroquine	II	CINJ, NCI	NCT00726596	Autophagic Cell Death in Patients with Hormone-Dependent Prostate-Specific Antigen Progression after Local Therapy for Prostate Cancer
Multiple myeloma, plasma cell neoplasm	Bortezomib hydroxy-chloroquine	I/II	University of Pennsylvania, NCI	NCT00568880	A Phase I/II Trial of Hydroxychloroquine Added to Bortezomib for Relapsed/Refractory Myeloma
Brain, central nervous system tumors	Hydroxy-chloroquine temozolomide	I/II	University of Pennsylvania, NCI	NCT00486603	A Phase I/II Trial of Hydroxychloroquine in Conjunction with Radiation Therapy and Concurrent and Adjuvant Temozolomide in Patients With Newly Diagnosed Glioblastoma Multiforme
Breast cancer	Hydroxy-chloroquine ixabepilone	I/II	CINJ, NCI	NCT00765765	Phase I/II Study of Ixabepilone in Combination with the Autophagy Inhibitor Hydroxychloroquine for the Treatment of Patients with Metastatic Breast Cancer
Lung cancer	Bevacizumab carboplatin hydroxy-chloroquine paclitaxel	I/II	CINJ, NCI	NCT00728845	Modulation of Autophagy with Hydroxychloroquine in Combination with Carboplatin, Paclitaxel and Bevacizumab in Patients with Advanced/Recurrent Non-Small Cell Lung Cancer -A Phase I/II Study

Adult solid tumors	Hydroxy-chloroquine temozolomide	I	University of Pennsylvania, NCI	NCT00714181	A Phase I Study of Hydroxychloroquine in Combination with Temozolomide in Patients with Advanced Solid Tumors
Non-small cell lung cancer	Gefitinib, hydroxy-chloroquine	I/II	National University Hospital, Singapore, Massachusetts General Hospital	NCT00809237	A Phase II with a Lead in Phase I Study to Examine the Tolerability, Safety Profile and Efficacy of Hydroxychloroquine and Gefitinib in Advanced Non-Small Cell Lung Cancer
Advanced cancer	Hydroxy-chloroquine, sunitinib malate	I	CINJ, NCI	NCT00813423	Anti-Angiogenic Therapy in Patients with Advanced Malignancies: A Phase I Trial of Sunitinib and Hydroxychloroquine
B-cell chronic lymphocytic leukemia	Hydroxy-chloroquine	II	North Shore Long Island Jewish Health System	NCT00771056	Autophagic Modulation with Phase II Study to Evaluate the Tolerability and Efficacy of Treatment of Previously Untreated B-Cell Chronic Lymphocytic Leukemia (B-CLL) Patients with Hydroxychloroquine
Pancreas Cancer	Hydroxychloroquine, Gemcitabine	I	University of Pittsburgh	NCT01128296	Phase I/II study of Preoperative Gemcitabine in Combination with Oral Hydroxychloroquine in Subjects with Resectable Stage IIb Or III Pancreatic Adenocarcinoma
Renal Cancer	Hydroxychloroquine	IA	University of Pittsburgh	NCT01144169	Neoadjuvant Study of Preoperative Hydroxychloroquine in Patients with Resectable Renal Cell Carcinoma
NOTE: Study citations were obtained from the National Institutes of Health (NIH)/NCI clinicaltrials.gov website Abbreviations: CINJ, The Cancer Institute of New Jersey; NCI, The National Cancer Institute.					

2.13 miRNAs as Biomarkers of RCC.

The modification of cell death pathways important in cancer progression were postulated by the DAMP Laboratory five years ago (42) but the causes of the shift, and its regulation, were obscure. One possible explanation may be the regulation by micro-RNAs (miRNAs). These are 18-22 bps long single stranded non-coding RNA that regulate gene expression in both plant and animals by interacting with and degrading or silencing as many as 100 separate messenger RNAs (mRNAs) (43-45). We have shown that tumor expression of miRNAs can limit immune recognition (51), and have focused on the receptor for advanced glycation end products (RAGE) (52) as a potential target for miRs (see below).

Role of miRNAs in Target Sensitivity to Lysis. miRNAs play an important role in cell differentiation, tumor progression, organogenesis and embrybogenesis. Many miRNA machinery genes including Dicer, AGO1, AGO3, AGO4 are down-regulated in tumors and play a role in inflammatory cells (45-50). Various miRNAs have been identified to be involved in regulation of the cell cycle including lin-4 and let-7 controlling cell differentiation and proliferation, miR-14as an apoptosis suppressor, miR-1, miR-273, lys-6, miR-181, miR-375, miR-143, and miR-196 for organogenesis. Further application of miRNA as gene therapies to deliver tumor suppression as

miRNA, anti-miRNA oligonucleotides (AMOs), or cholesterol conjugated AMOs, so-called antagomirs, are also in progress. Since miRNA plays an important role in cell differentiation and proliferation affecting many cell types including hematopoietic cells, it will be useful to understand the impact of miRNAs in the immune response in human biology (52-64).

Inhibition of miRNA processing by Dicer disruption up-regulates intercellular cell adhesion molecule (ICAM)-1 and enhances the susceptibility of colorectal tumor cells to antigen-specific lysis by cytotoxic T-lymphocytes (CTLs) whereas expression of other immunoregulatory proteins examined is not affected. Blockade of ICAM-1 inhibits the specific lysis of CTLs against Dicer-disrupted cells, indicating a pivotal role of ICAM-1 in the interaction between tumor cells and CTL. Both miR-222 and -339 are down-regulated in Dicer-disrupted cells and directly interact with the 3' untranslated region (UTR) of ICAM-1 mRNA (50). Modulation of Dicer or these miRNAs inversely correlated with ICAM-1 protein expression and susceptibility of U87 glioma cells to CTL-mediated cytolysis while ICAM-1 mRNA levels remain stable. Immunohistochemical and *in situ* hybridization analyses of 30 primary glioblastoma tissues demonstrated that expression of Dicer, miR-222, or miR-339 was inversely associated with ICAM-1 expression. We will similarly assess expression of ICAM-1 in circulating macrophages (52-59) activated by DAMPs in the context of this study.

Studies of miRNA in Renal Cancer. There have been several investigations of miRNA in renal cancer. miRNAs regulate renal development, activity and disease. Many kidney specific miRNAs and miRNAs specific to renal tumor vs normal tissue have been described (65, 66). Up-regulation of miR-28, miR-185, miR-27, and let-7f-2 has been identified in RCC relative to normal kidney (68). miR 141 and 200c are downregulated in RCC (69). Loss of miRNAs permits translation of proteins that promote tumorigenesis (67). An additional 38 miRNAs have been identified as increased in tumor, and 48 decreased assessed by microarray and PCR based techniques (70). miR-155 and miR-21 and miR-210 are overexpressed in clear cell carcinoma (71). miR-192 and miR-377 are associated with specific processes including matrix deposition whereas miR-200 and miR-205 regulate the epithelial-to-mesenchymal transition (65). The four histologic RCC types were effectively classified through assessment of miR 424, 203 and s-has-miR 33 (72). Identification of miRNA targets is still a challenge. Although there are methods to predict targets, few have been validated in mammals (66). New techniques available in our proteomic core may help advance this effort (73). miRNA can be successfully extracted from paraffin or frozen tissue (74). We recently completed our own studies of normal kidney, clear cell, oncocytoma, chromophobe, and papillary renal cell carcinoma (Bastacky SI, et al in preparation).

miR34c and miR214. We have identified two candidate miRNAs, miR34c and miR214 which are upregulated by HMGB1+, or HMGB1+/- tumor lysates respectively. HMGB1 is released from stressed or dying tumor cells in response to ischemia reperfusion injury and most therapeutic cytotoxic agent (55-59). HMGB1 (60-64) translocated to the cytosol in the setting of autophagy can bind Beclin-1 with dissociation of Beclin-1/Bcl-2. Mitochondrial HMGB1 regulates cellular bioenergetics and mitophagy by promoting phosphorylation and activation of ERK1/2 (pERK1/2). Reduced but not oxidized HMGB1 suppresses SOD and mTOR expression, and increases mitochondrial superoxide production, which in turn induces autophagy.

We have also identified putative miRNAs involved in HMGB1-induced signaling and/or differentiation including hsa-mir-155, hsa-miR-34c and hsa-mir-214 in three normal individuals (**Table 4)**. These are of particular interest to us due to their computationally calculated potential targets. Hsa-mir-155 is predicted to target Spi-1 (or PU.1) and TLR4, as well as MAP4K5 (a Mitogen-activated protein kinase), whereas hsa-34c is predicted to target Nemo/IKKγ of NFκB.

Both Spi-1 and IRF-8 are myeloid-specific transcription factors involved in monocyte activation and differentiation. The MAP Kinase may be involved in early HMGB1 signaling, which in turn may be regulated by miR-34c or miR214. With the peripheral blood obtained in this protocol, our next step will be to validate expression levels of these miRNAs by quantitative RT-PCR.

Table 4. Putative miRNAs involved in human myeloid cells stimulated with PAMPS/DAMPS

microRNA	FDR[1]	Geom. mean of intensities (KO lysate)	Geom. mean of intensities (WT lysate)	Geom. mean of intensities (LPS)	p-value	
					Permutation	Parametric
miR 155	0.18	1.36	1.45	3.97	$4.2x10^{-3}$	$4.6x10^{-4}$
miR-34c	0.28	1.39	6.71	1.14	$6x10^{-4}$	$1.4x10^{-3}$
miR-214	0.62	13.27	28.9	1.56	$3.7x10^{-3}$	$4.8x10^{-3}$

RCC

PBMCS IL-2 miRs.

We would also like to predict IL-2 responsiveness by miR profiling. Recent studies have shown (75, 76) that miRs (miR-146a, miR-182) are differentially expressed following treatment with IL-2 or during T-cell activation in mouse models. Those miRs involved in regulating genes involved in the IL-2 production pathway (e.g. NFAT, AP-1 and NF-κB) and controlling FOXO1 expression and STAT5 signaling pathway are of particular interest. In our preliminary data, 9 miRNAs are differentially expressed during IL-2 treatment in a dose dependent manner *in vivo*. (5 miRs down-regulated and 4 miRs are up-regulated). We propose to perform miR profiling of clinical blood samples from RCC patients before receiving IL-2. After a clinical response has been documented, the miR profiles from responders and non-responders will be used to generate a training set to predict IL-2 responsiveness.

2.14 Natural Killer (NK) Cells in Renal Cancer.

The role of NK cells in RCC has been considered possible since our earliest clinical trials of IL-2 demonstrated remarkable expansion of NK cells in the peripheral blood and tissues during therapy in mice and humans. We now know that RCC can attract various effector cells of both the innate and adaptive immune system, including natural killer (NK) cells, neutrophils (77), γδ T cells, NKT cells, dendritic cells (DC), and regulatory T cells (Tregs). These, histology, and serum factors predict clinical response (78-83). These individual cell types do not act in isolation but function within complex networks (84). Unlike melanoma, where a clear role for T-cells has been identified, the case for T-cells in successful immunotherapy of renal cell cancer is far less clear (85). Although RCC responds to immune modalities, T-cell therapies have shown a very limited success in contrast with melanoma. RCC immunotherapy with NK cell infusions and agents that enhance cell lysis is an intriguing alternative strategy (86). Several defects in cell-mediated immune function are apparent in RCC patients at the time of diagnosis. These defects transiently worsen after nephrectomy but return to baseline by post-resection on day 8 (87). High dose Interleukin 2 (HDIL-2) is FDA-approved for therapy of patients with metastatic melanoma and renal cell carcinoma,

based on its ability to induce durable responses in 5-10% of patients but associated with substantial side effects (88). Efforts to develop more potent IL-2 analogues that preferentially target activated T-cells but not NK cells have been made. BAY 50-4798, such an IL-2-specific agonist, was tested in patients with RCC (89) with only 1/20 responding, suggesting with such limited response, that NK cells are possibly important in this disease.

There are many infiltrating cells within RCC (90). When examining resected RCC, two subgroups are identified with high (>20% of the lymphocytes, n = 14), or low (<20%, n = 20), NK cell numbers. Although these cells are noncytolytic the majority of NK cells from tumors with high NK cells become cytotoxic following IL-2 exposure (113) whereas those from the low NK tumors do not. Significantly increased numbers of intratumoral $CD56^+$ NK cells ($p=0.008$) and $CD8^+$ T cells ($p=0.019$) compared with baseline are found after treatment with IL-2 and histamine (91). Low-dose IL-2, IFNα and GM-CSF, administered perioperatively (92) increased intratumoral numbers of $CD3^+$ T cells, $S100^+$ DC, $CD83^+$ DC and $IL\text{-}2R^+$ cells (4-fold, 2-fold, 10-fold and 20-fold, respectively, compared to controls). It further increased $TNF\alpha^+$ $CD8^+$ T cells and DC-SIGN $(CD209)^+$, $CD83^+$, $CD80^+$, $IL\text{-}12^+$ and DC-CK1 $(CCL18)^+$ DC. NK cells comprise a significantly higher proportion of in RCC TILs relative to peripheral blood colorectal or breast cancers, where these cells are found in small numbers. Notably, significantly fewer NK cells are observed in peripheral blood from patients with metastatic RCC (93). All the RCC lesions (n = 140) tested in one study by tissue microarray are cytosolic MHC class I-Related Chain A/B [$MICA/B^+$] (94), important for recognition by NKG2D expressing NK cells. Substantial numbers of NK cells in RCC can be detected with an antibody to NKp46, exclusively expressed by all NK cells in mouse and man. Cytotoxicity is observed after overnight activation with low-dose IL-2, proportional to the number of NK cells. Infiltrating NK cells express a functional inhibitory receptor, CD94/NKG2A and are cytolytic following cytokine stimulation (95).

Human TLR triggered monocytes up-regulate MICA and in turn downregulate NKG2D on autologous NK cells (96) and enhance production of IFN-γ. Thus myeloid cells (monocytes) and NK cells communicate directly during an innate immune response in humans. Two distinct activating NK cell synapses can be identified, a lytic and nonlytic one. Triggering NK cell proliferation and cytokine secretion is associated with NK cell 2B4, while macrophages are killed in NK cell NKG2D/DAP10 clusters (97). Interestingly, IFN-γ in turn down-regulates NKG2D ligand expression and subsequent cytolysis of MHC class I-deficient tumor cells by NK cells (98). This may be dependent on inducing a protective autophagy in these cells as we have shown in murine models. Expression of other MHC molecules on fresh RCC (99-101) such as HLA-G may be also be important for evasion from immune detection by NK cells. It is lost during cultivation of RCC cells and thus the tumor microenvironment and/or endothelium appear to be involved in its regulation, likely by IFNγ. HLA-G expression in the 27% of RCC lesions prevents lysis of RCC cells by cytokine immune effector cells. Enhancement of NK cytolytic activity by fever-range thermal stress is also dependent on NKG2D function (102). Assessment of pSTAT5 in patient PBMC in response to therapeutic IL-2 administration reveals persistent activation of STAT5 within circulating NK and T cells for prolonged periods (103). Although chloroquine is said to diminish cytolytic activity of NK cells, this has not been tested in patients with cancer or certainly those with renal cancer (104). We will examine the function of NK cells in the proposed blood samples obtained in this study.

2.15 Dendritic Cells (DCs) and Renal Cancer.

Multiple strategies have been employed to use DCs transfected with antigen or fused with tumor to generate an effective antitumor response. To date, they have been largely disappointing. Interestingly IL-2 treatment during DC-based immunotherapy of patients with metastatic renal cancer (105) leads to a transient and 'massive' increase of circulating natural regulatory T-cells, perhaps explaining their limited effectiveness; we hope to evaluate their number in the setting of HCQ administration. Intranodal autologous tumor lysate-DC vaccines with IL- 2 and IFNα therapy in RCC patients led to objective clinical responses in 9/18 patients with three CRs. Pre-IP-10 serum levels might have helped predict outcome (106). We [WJS] found decreased numbers of myeloid and plasmacytoid DC in the peripheral blood of RCC patients and were found at large numbers in the tumor, where they displayed an immature phenotype (DC-LAMP) (107). In another study 27 patients with RCC were vaccinated with DCs loaded with either a cocktail of survivin and telomerase peptides or tumor lysate depending on their HLA-A2 haplotype, with low-dose IL-2. None of the patients had an objective response (108). Although not subjected to clinical trial, apoptotic RCC appeared to be better inducers of cross-presenting activity than necrotic cells (109). Another tumor lysate (TL)-pulsed DC study in patients with RCC, only 1/9 patients achieved a partial response (110). Electrofused allogeneic DC/autologous tumor-derived cells in patients with 2/20 patients demonstrated only a single PR by RECIST criteria (111). As in other epithelial tumors, resected RCC in 25 patients, using immunohistochemistry, higher CD83+ DC in the tumor predicted a better survival ($p = 0.0339$) (112). Patients with RCC vaccinated with Carbonic anhydrase-IXG250/MN (CA9) -peptide-pulsed mature DCs, showed no evidence for induction of CA9-peptide-specific immunity or any clinical responses (113). Allogeneic DCs with or without cyclophosphamide have also been tested in patients with RCC. Only 2/22 patients had mixed responses, both in the cyclophosphamide group (114). Thus we conclude that little has been gained in this disease by conventional DC therapies. Little is known about chloroquine effects on circulating or tumor DCs but it appears to enhance human CD8+ T cell responses against soluble antigens *in vivo* (115). We are aware of the important role of autophagy as a source of antigen for cross-presentation but consider that in the setting of established cancer that autophagy in the tumor is the more critical process needing to be blocked to enable effector NK and T cells.

2.16 T-cells and Renal Cancer.

Human renal cell cancer (RCC) is clearly responsive to immunotherapy. Clinical responses may be mediated by"non-specific" (e.g. NK cells) or "specific" MHC-class-I-restricted tumor-specific CD8+ T lymphocytes. Typically RCC progresses, however, despite significant infiltration of various lymphoid cells. We (116) examined freshly isolated RCC tumor-infiltrating lymphocytes (TIL) derived from seven RCC patients for cytokine expression by the polymerase chain reaction (PCR). Established RCC tumor cell lines derived from these RCC patients were negative for interleukin-2 (IL-2), IL-4, IL-10, and interferon gamma and found to be positive for tumor necrosis factor alpha (TNF α), IL-6, IL-1 β, granulocyte/macrophage-colony-stimulating factor (GM-CSF), and transforming growth factor beta 1 (TGFβ1) message as detected by PCR. An identical pattern of cytokine mRNA expression was identified in other long-term RCC lines and in normal human kidney cells upon culture, but not in two Wilms tumor cell lines tested. Short-term-, and long-term-established RCC lines, but not Wilms tumor lines, secreted substantial levels of GM-CSF, TNFα, IL-1 beta, and IL-6 as detected by enzyme-linked immunosorbent assay. Both RCC lines

and Wilms tumor lines secreted TGF β1. In comparison, normal kidney cells secreted IL-6 and GM-CSF, but not IL-1 β, or TFG β 1 under identical *in vitro* cell culture conditions. We applied PCR-based methods to characterize the cytokine mRNA expression pattern in immune cells infiltrating into renal cell cancer without the need for expansion of such effector cells *in vitro*. Examining freshly collected RCC TIL by PCR from patients with primary cell cancer, we could demonstrate that such cells, but not lympho-mononuclear cells harvested from normal human kidney tissue, typically exhibit IL-4 and IL-10 mRNA expression. Given that alterations in tumor autophagy would be predicted to affect the death rate of these cells and provision of Ag for crosspriming of specific T cells, we propose to evaluate the HLA-A2+ patient cohort for changes in polarized CD8+ T cell response to a range of RCC and tumor stromal-associated antigens (EphA2, EGFR, HEr2, MAGE-3/6, PDGFR, RGS5, VEGFR1/2, G250 (CA-IX), DLK1, TEM1, and HBB) (117-121) pre- vs. post-treatment with hydroxychloroquine in the peripheral blood. In particular, IFN-γ response would be prioritized, but we could also consider granzyme or TNF-α (Tc1) vs. IL-4/-5 or IL-10. It would be possible to do this by intracellular cytokine stain in concert with surface staining for CXCR3 and/or VLA-4, given the importance of these markers for the ability of Th1 cells to home to and infiltrate tumors (113, 122).

Relapsed/refractory NBL patients receiving the hu14.18-IL-2 IC (humanized anti-GD2 monoclonal antibody linked to human IL-2) in a Children's Oncology Group (COG) Phase II trial were genotyped for KIR and HLA to determine if KIR receptor-ligand mismatch were associated with anti-tumor response. Thirty-eight of 39 patients enrolled had DNA available for analysis; 24 were found to have autologous KIR/KIR -ligand mismatch; 14 were matched. Seven of 24 mismatched patients experienced either complete response or improvement of their disease following IC therapy. There was no response or comparable improvement of disease in patients who were matched. Thus KIR/KIR-ligand mismatch was associated with response/improvement to IC ($p = 0.03$). Thus response or improvement of relapsed/refractory neuroblastoma patients after IC treatment is associated with autologous KIR/KIR-ligand mismatch, consistent with a role for NK cells in this clinical response (123,124). The NK content of TILS can be predicted by transcript levels of NKp46, perforin, CX3CL1, and CXCR1 with normalization using qRT-PCR and are prognostic indicators of survival (125).

2.17 Rationale for the current trial

The rationale for combining the high dose bolus aldesleukin with hydroxychloroquine includes potential positive interactions on the immune regulatory side, non-overlapping toxicities, and potential for prolongation and increased number of responses based on murine studies conducted at the University of Pittsburgh. This study is a multi-center phase II study designed to estimate the efficacy of combination therapy of standard high dose bolus IL-2 and various doses of hydroxychloroquine therapy in metastatic RCC patients. The combination of high dose bolus IL-2 and hydroxychloroquine has not been used in prior clinical trials. Hydroxychloroquine has been shown to be safe in other acute stressful situations such as operative procedures (127-130). Combinations of IL-2 with chloroquine have recently been shown in Pittsburgh to be statistically more effective than IL-2 alone, tested in three murine models (131). Based on this murine data, we could expect to double the complete response rate in patients. Use of the number of doses of IL-2 as a composite of maximum tolerated dose and dose limiting toxicity helps us (132, 133) deal with assessing the tolerability of the addition of a second drug to IL-2 designed to enhance efficacy

or decrease toxicity. Evaluating toxicity after 8 doses allows us to compare comparable amount of drug across patients when all are expected to have received all eight of the doses.

The primary outcomes for this study will be overall survival, progression-free survival and response rate. A secondary analysis will compare outcomes between the trial patients and a historical control set matched according to the prognostic criteria described by Motzer *et al* (20) [lactate dehydrogenase (> 1.5 upper limit of normal), hemoglobin (< lower limit of normal), corrected calcium (>10mg/dL), Karnofsky performance status (< 80%) and time from initial RCC diagnosis to start of interferon-α therapy of less than one year were significant prognostic factors. Patient categories are no risk factors, 1 or 2 risk factors, or 3 or more] (20).

2.18 Early experience with the regimen.

The first five patients were treated with 600mg of Hydroxychloroquine with one patient deemed a complete response. Out of the 13 patients treated with combination HCQ/IL-2 therapy using 1200 mg of Hydroxychloroquine, two patients experienced pronounced hypotension and tachycardia. Furthermore, one patient died from pulmonary emboli, not attributed to therapy. The cardiac events are consistent with IL-2 toxicities, but were observed somewhat earlier in the course of treatment. For that reason we are accruing another 21 patients to assess response in addition to the first five patients at the lower 600mg/d dose.

3.0 ELIGIBILITY

3.1 Inclusion Criteria

3.1.i. Histologically confirmed metastatic renal cell carcinoma with predominantly clear cell histology.

3.1.ii. Have measurable disease by RECIST 1.1 criteria. For example, this would include tumor in the lung, liver, and retroperitoneum. Bone disease is difficult to follow and quantify and as a sole site would not be acceptable.

3.1.iii. Patients must be at least 4 weeks from radiation or surgery and recovered from all ill effects.

3.1.iv. Age ≥18 years.

3.1.v. Karnofsky Performance Status ≥80%.

3.1.vi. Adequate end organ function:

a. Hematologic: ANC ≥ 1000 cells/μL, platelets ≥ 100,000/μL, hemoglobin ≥ 9g/dl (pre transfusion values used for prognostic factor, can be transfused or use recombinant erythropoietin growth factors but must not have active bleeding).

b. Liver: AST ≤ 2 x ULN (upper limit of normal), serum total bilirubin ≤ 2 x ULN (except for patients with Gilbert's Syndrome).

c. Renal: serum creatinine ≤ 1.5 mg/dL or estimated creatinine clearance ≥ 60ml/min using Crockcroft-Gault estimation **using the formula**

$$eC_{Cr} = \frac{(140 - \text{Age}) \times \text{Mass (in kilograms)} \times [0.85 \; if \; Female]}{72 \times \text{Serum Creatinine (in mg/dL)}}$$

This formula expects weight to be measured in kilograms and creatinine to be measured in mg/dL, as is standard in the USA.

d. Pulmonary: FEV1 ≥ 2.0 liters or ≥ 75% of predicted for height and age. (PFTs are required for patients over 50 or with significant pulmonary or smoking history defined as >20 pack years or history of COPD/emphysema).

e. Cardiac: No evidence of congestive heart failure, symptoms of coronary artery disease, myocardial infarction less than one year prior to entry, serious cardiac arrhythmias, or unstable angina. Patients who are over 40 or have had previous cardiac disease will be required to have a negative or low probability cardiac stress test for cardiac ischemia.

3.1.vii. Women should not be lactating and, if of childbearing age, have a negative pregnancy test within two weeks of entry to the study.

3.1.viii. Appropriate contraception in both genders (i.e., use of contraceptive strategies that prevent pregnancy; this most effectively would involve abstinence during the period of treatment but could include contraceptives, contragestives, and barrier methods.)

3.1.ix. The patient must be competent and have signed informed consent.

3.1.x. CNS: No history of cerebrovascular accident, transient ischemic attacks, central nervous system or brain metastases.

3.2 Exclusion Criteria

3.2.i. Patients who have previously received IL-2 are not eligible. Patients on HCQ in neoadjuvant protocols or in the past for clinical indications ARE eligible, as are patients who have previously received CTLA-4 and/or PD-1/PD-L1 antibodies.

3.2.ii. Concomitant second malignancy except for non-melanoma skin cancer, and non–invasive cancer such as cervical CIS, superficial bladder cancer without local recurrence or breast CIS.

3.2.iii. In patients with a prior history of invasive malignancy, less than five years in complete remission.

3.2.iv. Positive serology for HIV, hepatitis B or hepatitis C

3.2.v. Significant co-morbid illness such as uncontrolled diabetes or active infection that would preclude treatment on this regimen.

3.2.vi. Use of corticosteroids or other immunosuppresion (if patient had been taking steroids, at least 2 weeks must have passed since the last dose).

3.2.vii. History of inflammatory bowel disease or other serious autoimmune disease. (Not including thyroiditis and rheumatoid arthritis). Patients already on hydroxychloroquine for such disorders are not eligible.

3.2.viii. Patients with organ allografts.

3.2.ix. Uncontrolled hypertension (BP >150/100 mmHg).

3.2.x. Proteinuria dipstick > 3+ or ≥ 2gm/24 hours.

3.2.xi. Urine protein:creatinine ratio ≥ 1.0 at screening.

3.2.xii. Major surgery, open biopsy, significant traumatic injury within 28 days of starting treatment or anticipation of need for major surgical procedure during the course of the study.

3.2.xiii. Minor surgical procedures, fine needle aspirations or core biopsies within 7 days prior to starting treatment. Central venous catheter placements are permitted.

3.2.xiv. History of abdominal fistula, gastrointestinal perforation, or intraabdominal abscess within 6 months prior to starting treatment.

3.2.xv. Serious, non-healing wound, ulcer, or bone fracture.

3.2.xvi. History of tumor-related or other serious hemorrhage, bleeding diathesis, or underlying coagulopathy.

3.2.xvii. History of deep venous thrombosis, clinically significant peripheral vascular disease, or other thrombotic event.

3.2.xviii. Inability to comply with study and/or follow-up procedures.

3.2.xix. Individuals with known history of glucose 6 phosphate deficiency are excluded from the trial (possible issue with HCQ tolerance). No literature can be found to support hemolytic anemia having ever occurred in these patients and rheumatologists who frequently utilize this drug have not seen this as a side effect. Given that this has been a concern that has been raised, we believe it is prudent to exclude these patients with known G6PD deficiency from participation. These patients can receive IL-2 alone off trial.

3.2.xx. Patients with previously documented macular degeneration or diabetic retinopathy are excluded from the trial.

3.2.xxi. Baseline EKG with QTc > 470 msec (including subjects on medication). Subjects with ventricular pacemaker for whom QT interval is not measurable will be eligible on a case-by-case basis.

3.3 Minority and Women Inclusion

The patients for this study will be drawn from the oncology patient population seen at the Cytokine Working Group Institutions. No one will be excluded because of gender or race.

4.0 REGISTRATION PROCEDURES

To register a patient, the investigator or their designee will contact the Clinical Research Coordinators of Clinical Research Services (CRS) at the University of Pittsburgh Cancer Institute:

Deborah Hice, RN
Phone: 412-623-8962
Email: hiceda@upmc.edu

Kathy Mansfield, RN
Phone: 412-623-7048
Email: manskd@upmc.edu

If the coordinators are unavailable, contact Clare Grzejka, RN, BSN, at 412-623-4891 or grzejkac@upmc.edu. **Patients must be registered prior to the first dose of hydroxychloroquine (day -14).** The following information will be requested:

4.1 Investigator Identification

4.1.1 Institution name and/or affiliate

4.1.2 Investigator's name

4.2 Patient's Identification

4.2.1 Patient's initials and study ID

4.3 Eligibility Verification

Patients must meet all of the eligibility requirements listed in Section 3.0. Documents verifying eligibility, including a completed eligibility checklist, must be sent via fax or email to the coordinating center. **The coordinating center requires 24 hours to review eligibility of potential subjects from external sites.**

4.4 Cancellation Guidelines

No patient will be cancelled. Data will be collected on all patients, even if a patient does not receive protocol therapy. Reasons for a patient not initiating therapy should be submitted in writing as soon as possible. All patients receiving at least one week of IL-2 treatment and two weeks of oral HCQ will be included in the final evaluation.

4.5 Central Pathology Core

All patients will be required to have sent four representative unstained slides or paraffin blocks containing tumor. Consent for requesting pathology material will be required for enrollment in the study. This material will be batched and sent to the UPCI Pathology Tissue Bank Core for central pathology review.

All samples should be mailed by courier to:

UPCI Renal Cancer Tissue Bank
c/o Rajiv Dhir, MD
Department of Pathology
Shadyside Hospital
5230 Center Ave.
Pittsburgh PA 15232

5.0 PRE-STUDY TESTING/STAGING

All patients considered for entry in the trial should have measurable or evaluable metastatic disease as described in the RECIST 1.1 criteria (see Section 11.0). Staging will include:

5.1 History and physical examination within two weeks of the first day of treatment (day -14).

5.2 Within 4 weeks prior to treatment (day -14), the following procedures are required:

5.2.i CT scans of the chest/abdomen/pelvis

5.2.ii Brain MRI or enhanced brain CT

5.2.iii Bone scan (if clinically indicated)

5.2.iv Cardiac stress test: All patients over 40 years of age or with significant cardiac history

5.2.v Pulmonary function tests within 4 weeks of starting therapy for all patients over 50 years old or with significant pulmonary or smoking history

5.2.vi Cardiac ECHO or MUGA scan to assess LVEF

5.2.vii 12-lead EKG

5.3 Within 2 weeks prior to treatment (day -14) the following procedures are required:

5.3.i Urine protein:creatinine ratio (see appendix A)

5.3.ii Urinalysis

5.3.iii Laboratory tests: HIV, hepatitis B and C serology, pregnancy test when indicated, CBC with differential, comprehensive metabolic panel (creatinine, BUN, albumin, total protein, glucose, electrolytes, liver function tests [total bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase]), direct bilirubin, LDH, Ca, PO4, TSH, PT, PTT. A serum sample for determination of serum VEGF/ANG1/ANG2 levels will be drawn on day -14, prior to the dose of hydroxychloroquine.

5.4 Within 4 weeks after enrollment, tissue blocks or four unstained slides of renal tissue sent to central pathology facility.

6.0 TREATMENT PLAN & SCHEMA

6.1 Classification:

Patients will be classified by the following criteria prior to entry and the historical cohort will be group-matched according to these criteria. (Each criterion is dichotomized as absent - 0 or present -1):

- lactate dehydrogenase > 1.5 upper limit of normal = 1
- hemoglobin < lower limit of normal = 1
- corrected calcium (>10mg/dL) (total calcium – 0.707∗[albumin-3.4]) = 1
- Karnofsky performance status < 80% = 1
- time from initial RCC diagnosis to start of protocol therapy < 1 year = 1

Patients		# of Risk Factors	Median time to Death
Good Prognosis	=	no risk factors	30 months
Intermediate prognosis	=	1 or 2 risk factors	14 months
Poor prognosis	=	3 or more	5 months

6.2 Protocol Design

Patients will receive high dose IL-2 + hydroxychloroquine

Treatment: One course of treatment (84 days) will consist of high dose bolus IL-2 administered intravenously every 8 hours on days 1-5 (or 6) and 15-19 (or 20) (maximum 14 doses/5 days of administration) and hydroxychloroquine daily starting two weeks before IL-2 therapy and continued through up to three courses. Dependent upon when Dose 1 is administered on Day 1/Day 15, Dose 14 could require hospitalization on Day 6/Day 20.

6.3 Agents

6.3.1 Hydroxychloroquine

6.3.1.1 Administration.

Patients will receive HCQ every day starting at 14d before the first dose of IL-2. HCQ can be taken at any time of the day prior to or after meals. Tablets of HCQ are available in 200 mg strengths. HCQ will be administered in divided doses (BID) for doses above 200 mg po qd to minimize nausea. The lower dose (200) should be taken in the AM and the higher dose (400) at night due to the potential for GI distress. Patients should be told to swallow the whole capsule in rapid succession without chewing. Missed and vomited doses of HCQ can be skipped during therapy if necessary. The two-week lead in and long half-life of HCQ allow for this. Two dose reductions (Levels -1 and -2 in Table 5) may be allowed in a patient if treatment-limiting toxicity (Section 9.2) is observed. A pill diary will be provided and is required.

Table 5. Hydroxychloroquine Dose Schema

Dose Level	Dose hydroxychloroquine (mg/day)
1	600 mg
-1	400 mg
-2	200 mg

6.3.1.2 Description

- Generic name: Hydroxychloroquine sulfate
- Commercial name: Plaquenil
- Chemical name: 7-Chloro-4-[4-[ethyl-(2-hydroxyethyl)amino]-1-methylbutylamino] quinolon
- Supplied by: Commercially available; Sanofi-Aventis

6.3.1.3 Known Potential Toxicities of Hydroxychloroquine

- Central nervous system: Irritability, nervousness, emotional changes, nightmares, psychosis, headache, dizziness, vertigo, seizure, ataxia, lassitude.
- Dermatologic: Bleaching of hair, alopecia, pigmentation changes (skin and mucosal; black-blue color), rash (urticarial, morbilliform, lichenoid, maculopapular, purpuric, erythema annulare centrifugum, Stevens-Johnson syndrome, acute generalized exanthematous pustulosis, and exfoliative dermatitis).
- Gastrointestinal: Anorexia, nausea, vomiting, diarrhea, abdominal cramping, pancreatitis.
- Hematologic: Aplastic anemia, agranulocytosis, leukopenia, thrombocytopenia, hemolysis (in patients with glucose-6-phosphate deficiency).
- Hepatic: Abnormal liver function/hepatic failure (isolated cases).
- Neuromuscular & skeletal: Myopathy leading to progressive weakness and atrophy of proximal muscle groups (may be associated with mild sensory changes, loss of deep tendon reflexes, and abnormal nerve conduction).
- Ocular: Disturbance in accommodation, keratopathy, corneal changes/deposits (visual disturbances, blurred vision, photophobia - reversible on discontinuation), macular edema, atrophy, abnormal pigmentation, retinopathy (early changes reversible - may progress despite discontinuation if advanced), optic disc pallor/atrophy, attenuation of retinal arterioles, pigmentary retinopathy, scotoma, decreased visual acuity, nystagmus.

6.3.2 Interleukin 2

6.3.2.1 Administration

IL-2- 600,000 IU/kg IV bolus q 8 hrs beginning on day 1 and on day 15, for a maximum of 14 doses per cycle.

6.3.2.2 Description

Aldesleukin is recombinant formulation of interleukin-2 (IL-2). Aldesleukin is a nonglycosylated biosynthetic interleukin-2 (also known as T-cell growth factor), which differs only slightly in amino acid sequence from the natural compound with a serine for cysteine substitution at position 125 to allow correct folding. Aldesleukin is commercially available from Prometheus.

6.3.2.3 Mechanism of Action

Aldesleukin's effects are essentially identical to those of endogenous interleukin-2. Aldesleukin interacts with the high-affinity IL-2 receptor expressed on cells of the immune system and stimulates a cytokine cascade involving various interferons, interleukins, and tumor necrosis factors. Aldesleukin along with other cytokines induce proliferation and differentiation of B and

T-cells, monocytes, macrophages, and cytotoxic lymphocytes which include natural killer (NK) cells, cytotoxic T-cells, tumor-infiltrating lymphocytes (TIL), and lymphokine-activated killer (LAK) cells. Aldesleukin's antitumor activity is believed to result from activation of cytotoxic lymphocytes, however, the exact mechanism is unknown. Whether aldesleukin acts directly or through second messengers is also unclear, however, aldesleukin does elevate production of interleukin-1, tumor necrosis factors α and β, interferon γ, and interleukin-6.

6.3.2.4 Pharmacokinetics

Aldesleukin is administered parenterally. Following a short IV infusion, the drug is rapidly distributed to the extravascular and extracelluar space as well as to the liver, spleen, kidneys, and lungs. Approximately 30% of an administered dose is distributed within the plasma. The pharmacokinetics of aldesleukin may be affected by sodium dodecyl sulfate, the solubilizing agent in the commercial formulation. In addition, subcutaneous administration with albumin produces slightly higher and more prolonged serum concentrations of aldesleukin. Following distribution, aldesleukin is cleared from the systemic circulation by the kidneys through both glomerular filtration and peritubular extraction. The drug is then metabolized to amino acids by renal cells lining the proximal convoluted tubules. Very little drug is excreted unchanged in the urine. Following a 5 minute IV infusion, the serum distribution and elimination half-life in cancer patients was 13 and 85 minutes, respectively.

6.3.2.5 Toxicities

Abdominal pain, alopecia, anemia, angina, anorexia, anuria, arthralgia, ascites, atrial fibrillation, azotemia, back pain, chills, cholestasis, conjunctivitis, constipation, diarrhea, drowsiness, dysgeusia, dyspepsia, dyspnea, dysuria, edema, elevated hepatic enzymes, eosinophilia, erythema, exfoliative dermatitis, fatigue, fever, GI bleeding, GI perforation, glomerulonephritis, hallucinations, headache, hematuria, hepatomegaly, hyperbilirubinemia, hyperkalemia, hypoalbuminemia, hypocalcemia , hypokalemia, hypomagnesemia, hyponatremia, hypophosphatemia, hypotension, hypovolemia, infection, injection site reaction, interstitial nephritis, jaundice, leukocytosis, leukopenia, lymphopenia, malaise, metabolic acidosis, metabolic alkalosis, myalgia, myocardial infarction, nausea/vomiting, oliguria, pancreatitis, paranoia, petechiae, pleural effusion, premature atrial contractions (PACs), premature ventricular contractions (PVCs), proteinuria, pruritus, pulmonary edema, purpura, sinus bradycardia, sinus tachycardia, splenomegaly, stomatitis, supraventricular tachycardia (SVT), syncope, tachypnea, thrombocytopenia, thrombosis, urticaria, visual impairment, weakness, weight gain, weight loss, wheezing, xerosis.

6.4 Response Assessment/ Duration of Therapy

Patients will undergo CT scans at week 8 and week 12 (+/- 1 week) following initiation of IL-2 therapy and then after each course (every 10-12 weeks). After the final course (week 36), patients will undergo CT scan evaluation every 12 weeks (+/- 2 weeks) for up to one year or until tumor progression (whichever comes first). If additional scans are performed for clinical reasons, the study schedule may be altered to perform the requisite scans at a more acceptable time point. The change in timing of study scans must be approved by the local PI and documented. For patients who have not shown progression at the end of one year, response will be captured during follow-up for survival (explained below).

Patients with evidence of tumor shrinkage with all lesions measurable and evaluable may receive up to 3 courses of IL-2 therapy. Course 2 will start on or within 2 weeks following day 85 and follow the same schedule as in Course 1. Course 3 can start within 2 weeks of completion of Course 2. Patients not receiving IL-2 during course 2 with tumor shrinkage by week 24 can receive a 2nd course of IL-2 beginning within 2 weeks of week 25.

Patients with no evidence of progression by RECIST 1.1 criteria mayreceive continued treatment with hydroxychloroquine through one year of initiation of HCQ treatment (Day -14) or until disease progression.

Tumor response will be assessed by the NCI's Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 guidelines. Patients will undergo tumor evaluation with physical exam and CT scans at week 8 (+/- 1 week) and week 12 (+/- 1 week), then after each course (every 10-12 weeks). After the final course (week 36), patients will undergo CT scan evaluation every 12 weeks (+/- 2 weeks) for up to one year or until tumor progression (whichever occurs first). If additional scans are performed for clinical reasons, the study schedule may be altered to perform the requisite scans at a more acceptable time point. The change in timing of study scans must be approved by the local PI and documented. For patients who have not shown progression at the end of one year, response will be captured during follow-up for survival, which will be done on all patients via telephone contact and medical record review. Patients with progressive disease by RECIST 1.1 criteria will be taken off study.

Table 6. Tumor Assessment

Prior to therapy	**At week 8 (course 1), and at the end of courses 2 and 3.**	**Every 3 mos. until 1 yrs**	**At disease progression**
CT C/A/P, BS*, MRI**	CT C/A/P, BS*, MRI**	CT C/A/P, BS*, MRI**	CT C/A/P, BS*, MRI**
PE	PE	PE	PE

CT C/A/P = Computerized Tomography of the chest/abdomen/pelvis, BS = Bone scan, MRI = Brain MRI or enhanced Brain CT, PE = Physical examination.

Tumor evaluation will include CT of chest/abdomen/pelvis.

*Bone scan should be performed if bone metastases documented prior to study enrollment or if clinically indicated.

**Brain MRI or enhanced Brain CT is required prior to therapy for evaluation of brain metastases. The Brain MRI or enhanced Brain CT will be repeated if clinically indicated.

Response criteria are provided in section 10.0.

6.4.1 Toxicity Evaluation

Patients enrolled in this study will be evaluated clinically and with standard laboratory tests before and at regular intervals during their participation in this study. Safety evaluations will consist of medical interviews, recording of adverse events, physical examinations, blood pressure, and laboratory measurements. Patients will be evaluated for adverse events (all grades), serious adverse events (SAE), and adverse events requiring study drug interruption or discontinuation at each study

visit for the duration of their participation in the study. Specific monitoring procedures are as follows:

- During IL-2 treatment, AEs will be assessed daily.
- In patients with bleeding, hemostasis evaluation should be performed as clinically indicated
- All patients will be followed by either history, physical examination, laboratory tests or telephone interviews until resolution of AEs. The frequency of these contacts will depend on the type and severity of the SAE and will be determined by the investigator.
- Patients will have at least one telephone contact between cycles of IL-2 administration to assess toxicity. A CBC and complete metabolic survey will also be obtained.

All patients on trial will have a compilation of treatment (number of doses), toxicity scoring, laboratory results, and response assessment monthly (see Section 8) by the Clinical Trials Office and this information made available at the time of the monthly teleconferences, the Cytokine Working Group teleconferences, and to the local oversight DSMB. In addition, the trial will be monitored monthly by the UPCI Multi-Center Trial DSMC, which operates under the direct supervision of the UPCI DSMC. Data on accrual and adverse events at all sites will be aggregated by UPCI Clinical Research Services so that the Multi-Center DSMC has the necessary data to perform its function in a timely fashion.

Patients discontinued from the treatment phase of the study for any reason will be evaluated ~30 days (28-42 days) after the decision to discontinue treatment.

6.4.2 Clinical Assessment and Duration of Therapy

All patients will have blood tests and a thorough physical examination prior to each cycle of IL-2. Further therapy will be withheld until laboratory values and performance status return to within the eligibility criteria. IL-2 may be re-initiated once serum creatinine reaches ≤ 1.8 mg/dL (at the discretion of the treating physician).

Both responding and non-responding patients will be followed until death or final closure of the study. After the final course of treatment, follow-up procedures will be performed every 3 months (+/- 2 weeks) for up to one year or until disease progression (whichever occurs first). Follow-up procedures will include CT scan, routine laboratory tests, and physical examination. Subjects will then be followed for survival by telephone contact and medical record review every 3 months for one year following the time of disease progression or after one year off of treatment, then every 6 months for the next two years, then annually until death. A window of +/- 2 weeks is permitted. This will include review of any routine procedures performed as part of the patient's standard care (e.g., routine scans, physical examinations, laboratory exams, etc.). Attention will be paid to the time of disease progression and time of initiation of alternate therapies.

In the absence of treatment delays due to adverse events, treatment may continue for up to 3 courses per protocol or until one of the following criteria applies:

- Disease progression (see section 10.0),
- Intercurrent illness that prevents further administration of treatment,
- Treatment-related toxicities (see section 9.5),

- Subject decides to withdraw from the study, or is non-adherent,
- Subject becomes pregnant or begins breast-feeding,
- General or specific changes in the subject's condition rendering the subject unacceptable for further treatment in the judgment of the investigator.

Patients discontinued from the treatment phase of the study for any reason will be evaluated ~30 days (28-42 days) after the decision to discontinue treatment.

6.5 Ancillary Treatment

6.5.1 Venous Access Catheters

Institutional guidelines should be followed for venous access. However, it is recommended that patients with adequate peripheral venous access be treated through 2 large bore peripheral IVs or PICCs. Patients without adequate peripheral venous access should have a double lumen central venous catheter or PICC placed prior to initiating each course of therapy. Central venous catheters will be placed preferentially in the subclavian vein. **Infusaports should be used for neither IL-2 administration nor continuous IV hydration as this may create an unacceptable infection risk.** Catheters should be removed at the end of each hospital course. Patients requiring central venous catheters or PICC will receive antibiotic prophylaxis (e.g. cephaloxin 250 mg PO QID for those without penicillin allergies or ciprofloxacin, 250 mg PO BID, days 1-10 and 15-24 of each course). If catheter related bacteremia develops, the catheter should be removed and parenteral antibiotic treatment (vancomycin 1 gm IV q 12-24 hours depending on renal function) may be required.

6.5.2 Suggested Concurrent Therapy

Corticosteroids will not be permitted.

Antihypertensive therapy should be discontinued at least 48 hours prior to initiating each cycle of IL-2 and for the duration of the cycle. Investigators may choose to taper beta blockers over a few days prior to each cycle.

While IL-2 is administered (days 1-5 (or 6) and 15-19 (or 20)), patients may receive the following to abrogate toxicity related to IL-2 (start before IL-2 begins, continue for 12 hours after last IL-2 dose):

Acetaminophen 650mg PO every 4 hours.

Meperidine (25-50mg) IV or IM should be given in the case of severe rigors.

Indomethacin 25mg PO every 6 hours, or **Naprosyn** 750mg PO every 12 hours, or **Motrin** 600mg PO every 6 hours can be given for fever, chills and myalgias unresponsive to acetaminophen.

Ranitidine (150 mg) or **Axid** (150 mg) or **Pepcid** (20 mg) PO q 12 hours

for prophylaxis of gastrointestinal bleeding.

Lomotil or **Imodium** supplemented with paregoric, 10-14 ml PO q 4-6 hours PRN will be used for diarrhea.

Benadryl (25-50 mg PO),**Atarax** (25 mg PO), or **Gabapentin** (300-600mg PO TID) may be used for generalized erythematous skin rash and/or pruritus.

Furosemide (20-40 mg) IV/PO qd may be administered following completion of IL-2 infusion and until edema resolves and weight returns to baseline per local physician.

Keflex 250mg PO QID for prophylaxis of catheter-related infections.

7.0 LABORATORY STUDIES

The schedule for blood sampling is in Appendix C. Bloods will be sent directly by overnight courier to the UPCI for separation and cryopreservation.

UPCI – IMCPL
5117 Centre Avenue
1.26 Hillman Cancer Center
Pittsburgh, PA 15213
Attn: Processor
Phone: 412-624-0078

7.1 Baseline tumor samples All patients will be required to have four representative unstained slides or paraffin blocks containing tumor available to be sent within four weeks of enrollment in the study. This material will be batched and sent for analysis to:

UPCI Renal Cancer IL-2/HQ Tissue Bank
c/o Rajiv Dhir, MD; Department of Pathology; UPCI
5117 Centre Ave.
Pittsburgh, PA 15213

7.2 Serum and PBMC

a. All serum and plasma samples will be aliquoted and stored at -70 to -80ºC at the UPCI. Specimens will be sent overnight to the IMCPL/UPCI (see Appendix D):

b. Blood samples will be used for:

1) Western Blots and EM for measures of autophagy (LC3, p62, Caveolin 1, HMGB1, sRAGE)-Research Laboratory (DAMP Laboratory)
2) Serum angiogenic cytokine measurements including VEGF, ANG1, and ANG2; Fibronectin; Osteopontin (IMCPL)
3) Immune Function: TCR zeta chain, serum arginase/arginine levels (IMCPL)
4) Phenotype: KIRS, T cells, Treg, MDSC, NK cells, Measures of $CD11b^{+}IL4R\alpha^{+}$ myeloid derived suppressor cells, $CD56^{bright}$ and $CD56^{dull}$ NKp46+ NK cells, and $Lineage^{-}HLA\text{-}DR^{+}$ myeloid DCs, and $CD123^{+}Lineage^{-}$ plasmacytoid DCs will be assessed. Measures of cell surface RAGE and cytosolic RAGE on permeabilized cells will also be carried out. (IMCPL)
5) miRNA-Research Laboratory (DAMP Laboratory)
6) Bank DNA and/or RNA (i.e. RNA PAX tubes) for germline SNP, gene expression analysis etc.) See 7.5.

Autophagy assays will be run on all specimens

7.3 Correlate the level of autophagy in resected renal adenocarcinomas (where available) with measures of clinical outcome and response. We hypothesize that levels of autophagy in biopsies from patients on treatment will directly correlate with response to therapy. Fixed specimens in 10% formalin at room temperature, and the size and gross appearance of the tumors will be recorded. Select serial sections of the tumor specimens will be assessed for

clinicopathologic correlates, HMGB1, RAGE, LC3, Caveolin 1, and p62 staining as described below. The immunohistochemical staining results will then be analyzed statistically to examine the relationship between clinicopathological factors in the tumor, disease-free survival and overall survival.

Clinicopathological Correlates: 1) tumor size (≥3 vs <3 cm); 2) predominant differentiation of the tumor (well,moderately, or poorly differentiated); 3) lowest degree of tumor differentiation 4) angiolymphatic invasion 5) neural invasion; 6) margin status; 7) International.Union Against Cancer (UICC) pathological T and N stage.

Immunohistochemical staining for autophagy. We will use a modification of the technique described by Fujii et al to evaluate LC3 staining in tumor tissues. Briefly formalin-fixed, paraffin embedded tissue sections containing the maximal cancer tissue area will be processed for immunohistochemical staining. Because nerve cells stain for both RAGE and LC3 they will be used as internal positive controls for each section Tumor cells whose staining intensity is equal to or stronger than that of nerve cells will be judged to be strongly positive, whereas those whose staining weaker than that of the nerve cells will be scored as weakly positive. Tumor cells that do not stain positively for LC3 or RAGE immunohistochemically despite a positive internal control will be scored as negative. We will select sections from the center and the invasive border for independent review and scoring. The dominant intensity level of positive cells will be evaluated as follows: The level of intensity of staining in each area will be determined by the percentage of cells that stain negative, weakly positive, and strongly positive. When more than 50% of the positive cancer cells are strongly positive for LC3/RAGE in each area, the area will be scored as strongly positive, and when more than 50% of the LC3/RAGE positive cancer cells are weak positive the area will be designated weakly positive. When 30% of the cancer cells are weakly positive and 40% are strongly positive, the predominant intensity will be recorded as strongly positive. Specimens will then be classified into three groups according to the dominant overall intensity of the cancer tissue: negative, weakly positive, or strongly positive. A similar procedure will be carried out to assess HMGB1 staining of the cytosol. Two independent pathologists, blinded to the clinical outcomes will evaluate all of the immunohistochemical slides and their results compared and reviewed for discrepancies by a third party.

7.4 Correlate the levels of autophagic markers with measures of clinical outcome in peripheral blood mononuclear cells. We hypothesize that there will be biomarkers in the peripheral blood that will be specifically associated with induction of autophagy in renal cancer patients. We further hypothesize that these markers will correlate with treatment status, stage, and response to therapy, disease progression and overall survival. To test this hypothesis we will correlate the level of autophagy in the peripheral blood mononuclear cells with these measures of clinical outcome. Blood samples will be collected according to the schedule outlined in Table 6. Peripheral blood mononuclear cells are then separated from whole blood via ficoll hypaque separation and frozen in DMSO. Cells will be lysed for western blot analysis for RAGE, HMGB1, LC3, LC3-2, pre-LC3, caveolin 1, and p62. Levels of autophagic proteins, and RAGE/HMGB1 will then be compared between time points and correlated with tumor clinicopathologic correlates, response to therapy, time to progression, and overall survival.

7.5. Evaluation of KIR/KIR-ligand Genotype and Response to Therapy. KIR genotyping will be performed on patient DNA samples by PCR sequence-specific primer technique (SSP Unitray assay, Invitrogen Corporation, Carlsbad, CA) in the UPCI Genomics Facility. KIR-ligand typing

will be performed at low-resolution on the same samples for HLA-B & -C loci by reverse PCR-SSO methodology (LifeMatch assay, Gen-Probe, Inc, Stamford, CT) and for high-resolution HLA-C alleles by direct sequencing (AlleleSEQR assay, Abbott Labs, Des Plaines, IL). The four inhibitory KIR genes (KIR2DL1, KIR2DL2, KIR2DL3, KIR3DL1) evaluated in this study and their corresponding KIR-ligands (HLA-C1, HLA-C2, or HLA-Bw4). KIR/KIR-ligand mismatch will be defined as absence of one or more HLA alleles known to be ligands for the inhibitory KIR genes present, using previously published criteria.

8.0 DATA MANAGEMENT AND DATA SAFETY MONITORING PLAN

The number of IL-2 doses administered, severe adverse events, including their relatedness to HCQ (definitely, probably, possibly or unlikely), clinical response (Section 10.3), date of progression and date of death will be forwarded immediately to the Data Coordinating Center at UPCI for monthly presentation to the CWG and the UPCI Multi-Center DSMC. The UPCI IMCPL will report to the DCC on samples received on a monthly basis. Biomarker and laboratory data that will be evaluated at the end of the trial need only be reported to the DCC when the trial is closed to accrual, but more timely reporting is advisable so that missing and questionable data can be resolved prior to final data analysis. The DCC will use the UPCI Clinical Trials Management Application to store and manage all demographic, clinical, laboratory and biomarker data required to achieve the Specific Aims.

9.0 EVALUATION AND MANAGEMENT OF TOXICITY (DOSE MODIFICATION)

9.1 General

Investigator/Sub-investigators, regulatory, CRS management, clinical research coordinators, clinical research associates, data managers, and clinic staff meet regularly in disease center Data Safety Monitoring Boards (DSMB) to review and discuss study data to include, but not limited to, the following:

- serious adverse events
- subject safety issues
- recruitment issues
- accrual
- protocol deviations
- unanticipated problems
- breaches of confidentiality

All toxicities encountered during the study will be evaluated on an ongoing basis according to the NCI Common Toxicity Criteria version 4.0 and recorded prior to each course of the investigational therapy. Any modifications necessary to ensure subject safety and decisions to continue, or close the trial to accrual are also discussed during these meetings. All study data reviewed and discussed during these meetings will be kept confidential. These reviews are summarized by each respective disease center and submitted to the University of Pittsburgh Cancer Institute's Data and Safety Monitoring Committee (UPCI DSMC) for their monthly review of all disease centers' studies. The UPCI DSMC will provide oversight for this study. At the time of renewal a summary of all serious adverse events and modifications will also be reported to the UPCI DSMC.

Any modifications necessary to ensure patient safety will be submitted to the IRB prior to implementation. The IRB will be notified of any change in the risk/benefit ratio which would affect whether the study should continue. All protocol deviations or breaches of confidentiality will be reported to the IRB according to IRB guidelines. If any literature becomes available which suggests that conducting this trial is no longer ethical the study will be terminated and the IRB will be notified of the new findings in the form of an Unanticipated Problem submission. All study treatment associated adverse events that are serious, at least possibly related and unexpected will be reported to the IRB. DSMC reports and approval will be submitted to the IRB at the time of renewal.

All records related to this research study will be stored in a locked environment. Only the researchers affiliated with the research study and their staff will have access to the research records.

Safety data for each patient at the end of each cycle of IL-2 will be provided by all sites to the PI at UPCI.

Patients with life-threatening or persistent, severe toxic reactions to IL-2 will receive no further treatment with this agent. Specific toxicities, which require special consideration, are discussed below. Previously unknown or severe toxicities will be reported to the PI as Adverse Drug Reactions. They will also be reported to the FDA on FDA Form 3500A Medwatch report. A courtesy copy of these reports will also be forwarded to Prometheus Drug Safety Department. See section 12.0.

9.2 Dose Modification for Toxicity: HD IL-2 and Hydroxychloroquine

Modification of the treatment protocol will occur by withholding doses of IL-2 rather than continuing therapy at a reduced dose. The only dose modification for patients of Hydroxychloroquine will be for those individuals deemed by the treating investigator as 'likely' due to hydroxychloroquine or the combination and not to IL-2 alone based on their experience and dose reduction as shown in **Table 5**. Missed doses of IL-2 will not be made up. Dose of IL-2 will be withheld for:

9.2.1 Hypotension refractory to fluids and pressors or requiring unacceptably high pressor doses (those associated with individual decrease in extremity perfusion). We will use vasopressors judicially where necessary to support patients with limiting kidney/other organ perfusion. Requirement for vasopressors will be a relative indication for concluding a cycle (see 10.4.2.3 below).

9.2.2 Anuria for>24 hours and unresponsive to fluid replacement

9.2.3 Respiratory distress requiring oxygen>4 liters to maintain O_2 saturation>95%.

9.2.4 Confusion (mental status changes can progress to paranoia despite discontinuation of IL-2; it is imperative that the IL-2 be stopped at any sign of persistent confusion or disorientation).

9.2.5 Sustained ventricular tachycardia or any sign or symptom of myocardial ischemia or myocarditis. Patients experiencing sustained ventricular tachycardia or myocardial ischemia should not receive further treatment with IL-2

9.2.6 Metabolic acidosis with HCO_3<18, despite attempts to correct with IV HCO_3.

9.2.7 Atrial fibrillation or myocarditis.

9.2.8 Documented systemic infection.

9.2.9 Management of Nausea and Vomiting. The frequency of nausea and vomiting with interleukin 2 administration falls within a broad category of moderate emetic risk (NCCN Practice Guidelines, 2010). It may be enhanced by HCQ. Patients with nausea and vomiting will be given 8 mg bid of ondansetron (a 5-HT3 receptor antagonist). For nausea and vomiting that is not controlled with regimen of ondansetron a general principle (NCCN Practice Guidelines, 2010) is to add one agent from a different drug class to the current regimen 5-HT3 and NK1 receptor antagonists are very safe drugs that are standards of care in this area. Although not well studied, by carefully monitoring NV and using appropriate treatments patients might have better nutrition and maintenance of body weight. Records of antiemetics given routinely or as part of symptom management will be recorded.

or Any other serious toxicity that is not controlled at time of next dose.

9.3 Specific IL-2 Toxicity Management

Several treatment-related toxicities have been uniquely associated with the administration of high-dose IL-2. Antihypertensive medication should be stopped prior to IL-2 therapy.

Recommendations for management of the more significant toxicities typically seen with high-dose bolus IL-2 are as follows (the following are only optional guidelines for toxicity management):

9.3.1 Fluid Replacement. Excessive fluid replacement will increase the patient's likelihood of developing pulmonary edema. It is suggested that when intravenous access has been established; begin administration of normal saline at 75cc/hour IV. Once patients have gained greater than 15% of baseline weight, elective discontinuation of IL-2 is recommended. Diuretics should not be given unless severe symptomatic fluid retention develops and blood pressure is adequate.

9.3.2 Hypotension. Administration of high dose IL-2 leads to decreased peripheral vascular resistance and consequent hypotension. In order to manage this toxicity, the physician may utilize the following guidelines:

1. Monitor patients in a setting capable of providing level high intensity care.

2. Prior to starting IL-2 therapy determine a minimum tolerated blood pressure (MTBP). For patients under age 40 and with no prior history of ischemic or valvular heart disease, the MTBP can be a systolic blood pressure (SBP) of 80 mmHg, while the MTBP for all other patients should be a SBP of 85-90 mmHg based on perceived risk of cardiac toxicity.

3. When a patients systolic BP falls below the MTBP, suggested therapy may involve (in the following order):

a. Begin fluid boluses (250cc normal saline IV over 15 min. may repeat x 2) until SBP is > MTBP.

b. Should fluid boluses fail make the SBP>MTBP, vasopressor support such as phenylephrine could also be instituted (.1-2 ug/kg/min) to maintain SBP.

c. If blood pressure cannot be maintained on phenylephrine alone, or if unacceptable tachycardia develops, treatment with dopamine (1-6 ug/kg/min) should be added to sustain blood pressure.

9.3.3 Management of Arrhythmias/Myocarditis. If significant arrhythmias occur at any stage in the patient's treatment (whether on pressor agents or not), the possibility of myocardial ischemia/infarction must be excluded by both EKG and cardiac enzyme assessment. Patients who develop atrial fibrillation should have IL-2 doses held. Therapy may resume when the patient converts to normal sinus rhythm and is hemodynamically stable. If a significant supraventricular arrhythmia occurs while a patient is on dopamine therapy; neosynepherine should also be substituted for dopamine as initial blood pressure

support. Patients experiencing sustained ventricular tachycardia or documented myocardial ischemic episodes during therapy should not receive further treatment with IL-2. Patients with myocarditis may resume treatment in subsequent courses if CPK returns to normal. A cardiac ECHO documenting normal cardiac function should be performed prior to restarting therapy.

9.3.4 Management of Neurotoxicity. Doses are held rather than reduced for neurotoxicity. If Grade 4 neuro-cortical toxicity is encountered and is not reversible within 48 hours, no further treatment should be given and the patient should be removed from the study. If Grade 4 toxicity is reversible to Grade 1 within 48 hours, future treatment may be considered (in subsequent courses) if the patients shows any evidence of tumor regression and as discussed with the patient.

9.3.5 Metabolic Acidosis. In the course of IL-2 therapy, when a patient's HCO_3 falls to below 20, $NaHCO_3$ should be added to the maintenance IV infusion. Should the HCO_3 level fall below 18, IL-2 therapy should be held, and bolus infusions of $NaHCO_3$ should be instituted. IL-2 therapy may resume if repeat $HCO_3 > 18$. Sodium acetate may be substituted for $NaHCO_3$ per institutional policy.

9.3.6 Low Urine Ouput. Fluid boluses and 'renal' dose dopamine may be administered. Weight gains/edema may serve as a basis for holding doses.

9.3.7 Other Toxicities. Increases in the serum creatinine to 2.0-3.5 mg/dl and total bilirubin to 3.0-10.0 mg/dl are common and reversible upon cessation of treatment. Doses of IL-2 have not generally been withheld for renal and hepatic dysfunction alone. Although toxicity may become severe, recovery usually occurs following cessation of IL-2 and vigorous supportive care is warranted.

9.4 Management of Grade 4 Toxicity

Patients with Grade 4 (Life Threatening) toxicity (with the exception of those Grade 4 toxicities listed in Section 10.5) may be treated with dexamethasone 4 mg qid until side effects improve to an acceptable level as indicated. Two dose reductions of hydroxychloroquine (Levels -1 and -2 in **Table 5**) may be allowed in a patient if treatment-limiting toxicity considered 'likely' attributable to the combination (and not to IL-2 alone) by the treating investigator is observed. A pill diary will be provided and is required.

9.5 Toxicity Criteria for Discontinuing Treatment

Patients will not be considered for further therapy if the following toxicities are encountered:

9.5.1 Pulmonary toxicity requiring endotracheal intubation

9.5.2 Renal dysfunction requiring dialysis

9.5.3 Grade 4 cardiac dysrhythmia or Grade 2 or 3 dysrhythmia not easily controlled with medical management.

9.5.4 Myocardial ischemia (Grade 3 or 4) or infarction or symptomatic myocarditis (note:

asymptomatic CPK or CPK-MB band elevations without EKG changes are not a contraindication to further treatment).

9.5.5 Coma

9.5.6 Life-threatening sepsis

9.5.7 Pericardial tamponade

9.5.8 Bowel ischemia or perforation

9.5.9 Grade 4 hypertension or reversible posterior leukoencephalopathy syndrome (RPLS)

9.5.10 Grade > 2 pulmonary or CNS hemorrhage

9.5.11 Grade 4 hypertension or hemorrhage, symptomatic grade 4 venous thromboembolic event, nephrotic syndrome

9.5.12 Any grade arterial thromboembolic event

9.5.13 Grade 4 congestive heart failure

9.5.14 Wound dehiscence requiring medical or surgical intervention

9.5.15 Inability of subject to comply with study requirements

9.5.16 Any other severe or life-threatening toxicity which, in the opinion of the investigator, would preclude further treatment with these agents, or has not resolved substantially to an acceptable level as determined by the local PI.

Patients who have an ongoing Grade 4 or serious adverse event at the time of discontinuation from study treatment will continue to be followed until resolution of the event or until the event is considered irreversible (see Section 7.1.3).

10.0 DETAILED RESPONSE ASSESSMENT (also see 6.4)

Response will be defined by NCI's Response Evaluation Criteria in Solid Tumors (RECIST) 1.1.

10.1 Disease Evaluation

Measurable disease - the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

Measurable lesions - lesions that can be accurately measured in at least one dimension with longest diameter (longest diameter to be recorded) as ≥20 mm using conventional techniques or ≥10 mm with CT scan or ≥10 mm with calipers by clinical exam.
Malignant lymph nodes - to be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable lesions - all other lesions, including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis), i.e., bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, and also abdominal masses that are not confirmed and followed by imaging techniques. Non-measurable also includes lesions that are < 20 mm by chest x-ray

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes).

All measurements will be taken and recorded in metric notation, using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

10.2 Methods of Measurement

Conventional CT and MRI should be performed with cuts of 5 mm or less in slice thickness contiguously. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. Spiral CT should be performed using a 5-7 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen and pelvis.

Baseline documentation of "Target" and "Non-Target" lesions:

<u>Target Lesions</u>

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which

circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum of the diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target Lesions

All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of unequivocal progression of each should be noted throughout follow-up.

10.3 Response Criteria

Evaluation of target lesions

*Complete Response (CR):

Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm. For patients with > 90% Partial Response defined as at least a 90% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD sustained for greater than one year will be scored as a CR (maximal patient benefit), given the difficulty of evaluating lesions in some instances.

*Partial Response (PR):

At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD

* Minimal Response (MR):

At least a 20% decrease in the sum of the LD of target lesions, taking as reference the base line sum LD

*Progressive Disease (PD):

At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm.

* Stable Disease (SD):

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started. Note: a change of 20% or more that does not increase the sum of the diameters by 5 mm or more is coded as SD.

Evaluation of non-target lesions

* Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis)
* Stable Disease (SD): Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits
* Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. Although a clear progression of "non target" lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later on by the review panel (or study chair).

Evaluation of best overall response:
The best overall response is the best response recorded from the date of first protocol treatment until disease progression/recurrence (taking as reference for PD the smallest measurements recorded since the treatment started). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Table 7. Response Criteria

Target lesions	Non-Target lesions	Overall response
CR	CR	CR
CR	Incomplete response/SD	PR
PR	Non-PD	PR
MR	Non-PD	MR
SD	Non-PD	SD
PD	Any	PD
Any	PD	PD
Any	Any	PD

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration". Every effort should be made to document the objective progression even after discontinuation of treatment. In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

10.4 Confirmation

The main goal of confirmation of objective response is to avoid overestimating the response rate observed. To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval (in general, not less than 6-8 weeks) that is defined in the study protocol.

10.5 Duration of overall response

The duration of overall response is measured from the time measurement criteria are first met (i.e., not from time of confirmation of response) for CR or PR (whichever status is recorded first) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

10.6 Duration of stable disease

SD is measured from the date of first protocol treatment until the criteria for disease progression are met, taking as reference the smallest measurements recorded since the treatment started.

10.7 Time to Progression

The time to progression (TTP) is defined as the time from the date of first protocol treatment until the date disease progression criteria are met (in responding patients progression criteria uses the reference of the smallest measurements recorded since the treatment started) or is censored at date of last disease assessment for those who have not progressed.

10.8 Progression-free Survival

Progression-free survival (PFS) is defined as the time from date of first protocol treatment until the date disease progression criteria are met (in responding patients progression criteria uses the reference of the smallest measurements recorded since the treatment started) or date of death from any cause, or is censored at date of last disease assessment for those who have not progressed.

10.9 Survival:

Survival is calculated from the date of first protocol treatment to the date of death, or censored at date of last contact.

11.0 PARTICIPANTS EVALUABLE FOR RESPONSE

Reporting of results:

Clinical Results: All patients included in the study will be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories:

1) complete response
2) >90% partial response
3) partial response
4) stable disease
5) progressive disease
6) early death from malignant disease (within 3 months of initiating therapy)
7) early death from toxicity
8) early death because of other cause
8) unknown (not assessable, insufficient data).

All patients who met the eligibility criteria and receive at least one week of IL-2 treatment will be included in the main analysis of the response rate. Patients in response categories 5-8 will be considered as failing to respond to treatment (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate.

12.0 REPORTING OF ADVERSE REACTIONS

ADVERSE EVENT DEFINITIONS

Adverse event means any untoward medical occurrence associated with the use of the drug in humans, whether or not considered drug related.

Adverse reaction means any adverse event caused by a drug.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility (definitely or probably) that the drug caused the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than "adverse reaction"

Serious Adverse Event: Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Specifically, results in death, is life-threatening, requires inpatient hospitalization or causes prolongation of existing hospitalization, results in persistent or significant disability/incapacity, is a congenital anomaly/birth defect, or is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [e.g., medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Also, included: any subject death within 30 days of the last dose of study drug, regardless of the causality.

Scheduled protocol inpatient hospitalizations do not meet the criteria above for recording and reporting as an SAE. Only events which would prolong the scheduled inpatient stay to be resolved or unplanned hospitalizations due to events occurring between scheduled inpatient stays should be recorded as SAEs.

Life-threatening, suspected adverse reaction. A suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator (i.e., the study site principal investigator), its occurrence places the patient or research subject at immediate risk of death. It does not include a suspected adverse reaction that had it occurred in a more severe form, might have caused death.

Unexpected, suspected adverse reaction. A suspected adverse reaction is considered "unexpected" if it is not listed in the general investigational plan or clinical protocol; or is not listed at the specificity or severity that has been previously observed and/or specified.
All observed or volunteered adverse events (serious or non-serious) and abnormal test findings, regardless of study group or suspected causal relationship to the study drug(s) will be recorded in the subjects' case histories. For all adverse events, sufficient information will be pursued and/or obtained so as to permit 1) an adequate determination of the outcome of the event (i.e., whether the event should be classified as a *serious adverse event*) and; 2) an assessment of the casual relationship between the adverse event and the study drug(s).

Adverse events or abnormal test findings felt to be associated with the study drug(s) will be followed until the event (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the Principal Investigator.

In the event of an adverse event the first concern will be for the safety of the subject.

REPORTING OF SUSPECTED ADVERSE REACTIONS

In the event of any unexpected or serious suspected adverse reaction, the PI, the institutional review board (per institutional reporting requirements), and Prometheus Drug Safety Department will be notified using the FDA Form 3500 MedWatch report. We will report all secondary malignancies occurring or following treatment by this protocol.

All events meeting the definition of a serious adverse event should be recorded on a MedWatch 3500A Form:
(http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM048334.pdf)
and submitted to the Clinical Research Coordinators and Prometheus Laboratories' Drug Safety Department:

Deborah Hice, RN
Phone: 412-623-8962
Email: hiceda@upmc.edu
Fax: 412-623-8974

Kathy Mansfield, RN
Phone: 412-623-7048
Email: manskd@upmc.edu
Fax: 412-623-8974

Prometheus Laboratories' Drug Safety Department
Email:drugsafety@prometheuslabs.com
Fax: 858-754-3046.

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500 form:

Reporting of toxicity will include:

1) Treatment regimen (dosing frequency, combination therapy)
2) Protocol description (and number, if assigned)
3) Description of event, severity, treatment, and outcome, if known
4) Supportive laboratory results and diagnostics
5) CTCAE Version 4.0 Term and Grade of event
6) Investigator's assessment of the relationship (causality) of the adverse event to each investigational product and suspect medication

- the likelihood of the relationship between the toxicity and treatment (i.e., unrelated, unlikely, possibly, probably, or definitely related to treatment)
    - list alternate causality if event is unrelated or unlikely related to treatment (e.g. attributed to underlying disease, history of COPD, etc.)
- whether the side effect noted falls within the expected toxicity profile of the agent employed

Follow-up information:

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500 report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500 form
- Summarizing new information and faxing it with a cover letter including subject identifiers (i.e. D.O.B. initial, subject number), protocol description and number, if assigned, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted (The subject identifiers are important so that the new information is added to the correct initial report)

Occasionally Prometheus may contact the reporter for additional information, clarification, or current status of the subject for whom and adverse event was reported.

Assessing Causality:

Investigators are required to assess how likely it is that hydroxychloroquine caused or contributed to an adverse event (definitely, probably, possibly, or unlikely). The following factors should be considered when assessing relatedness.

- The temporal relationship of the clinical event to hydroxychloroquine administration
- The likelihood that IL-2 provides a sufficient explanation for the observed event.
- Other drugs, therapeutic interventions or underlying conditions that may provide a sufficient explanation for the observed event.

13.0 STATISTICAL ANALYSIS AND STUDY SIZE

13.1 Objectives and Design

The primary objective of this Phase II trial is to estimate the proportions of patients with metastatic RCC treated with high-dose IL-2 combined with hydroxychloroquine experiencing complete response, or any response. The secondary objectives are to compare response, time to progression and survival to historical data to determine the toxicity of the treatment regimen, to evaluate PD, and to examine correlative science endpoints. Disease endpoints are defined in Section 10.

The accrual goal is 39 participants. All study participants will receive the same high-dose IL-2 treatment, which is considered standard of care. The initial dose of HCQ will be 600 mg/day for all patients. If no limitations on additional dosing (treatment-limiting toxicities [TLTs], as defined in Section 9.2), dosing will continue at 600mg/d, otherwise, HCQ will de-escalate to 400 mg/d. Toxicity and response will be monitored after every three patients (Section 13.4), and will be stopped if the probability that treatment with IL-2+HCQ is excessively toxic or ineffective. The study may be opened more broadly to additional Cytokine Working Group sites whenever sufficient evidence has accrued that treatment with IL-2+HCQ is no more toxic or less effective than treatment with IL-2 alone.

13.2 Analysis Plan

The data will be analyzed for response, overall survival and other endpoints, as described in this section; data will only be analyzed as specified in Section 14.3.1 to ensure the toxicity of IL-2+HCQ is not unexpectedly high. All analyses will be performed using the most recent versions of SAS and/or R available.

13.2.1. *Estimate the proportion of patients with metastatic RCC treated with IL-2 combined with hydroxychloroquine at 600mg/d who experience a clinical complete response.* Response is defined in Section 10.3. The probability of each category of response will be estimated. Secondary analyses will employ cumulative logit regression for increasing levels of response, with adjustment for demographic and clinical covariates. All point estimates will be accompanied by appropriate 95% confidence intervals.

13.2.2. *Compare the response, overall survival and time to progression of patients with metastatic RCC treated with IL-2 combined with hydroxychloroquine to the published response of patients treated with high dose IL-2 alone*. Logistic and proportional hazards (Cox) regression will be used to compare clinical response, and OS and PFS, respectively, to examine the effect of HCQ on the efficacy of IL-2. Formal tests of non-inferiority are beyond the scope of this Phase II study, and these secondary analyses are not statistically powered.

13.2.3. *Characterize the toxicity of IL-2 combined with hydroxychloroquine in patients with metastatic RCC: number of doses of IL-2 administered during the first course of therapy; toxicity after the scheduled 9th dose of IL-2; frequency of grade III and IV or unexpected or rare toxicities*. Number of doses, maximal toxicity grade and incidence of treatment-limiting toxicities (TLTs, as defined in Section 10.5) will be tabulated.

13.2.4 *Evaluate the utility of known prognostic criteria for RCC patients on clinical outcome*. Prognosis is evaluated by whether the patient has 0, 1, or 2, or 3 or more of the following risk factors:

- lactate dehydrogenase (> 1.5 upper limit of normal)
- hemoglobin (< lower limit of normal)
- corrected calcium (>10mg/dL)
- Karnofsky performance status (< 80%)
- time from initial RCC diagnosis to start of interferon-α therapy of less than one year

This prognostic classification will be added to the baseline models described in Sections 13.2.1 to 13.2.3 to determine if any of the factors would significantly change the prognostic evaluation, and to the dynamic and post-treatment factors to potentially increase the statistical power.

13.2.5 *Correlate baseline laboratory parameters (miRNAs pre- and post-IL-2; KIR genotyping; NK enumeration in the peripheral blood; DC function, TCR-ζ chain expression, arginase or arginine levels, proposed pathological/molecular prognosis classification described in Section 2.6) with toxicity, response, and survival.* Stepwise proportional hazards or logistic regression will be used to add variables to the models resulting from the analyses in Section 13.2.4.

13.2.6 *Assess if changes in biomarkers measured in PBMC (Section 7.4) are associated with clinical response, overall survival or progression-free survival.* Stepwise proportional hazards or logistic regression will be used to determine if changes of any of the markers in Section 7.4 are related to patient outcomes, after adjusting for the prognostic criteria in 13.2.4.

13.3 Monitoring for Toxicity and Response

13.3.1 21 additional patients will be enrolled at IL-2+HCQ 600 mg/day on this revised protocol. If one or more TLTs are observed, treatment will be de-escalated to IL-2+HCQ 400 mg/day.

13.3.2 The number of overall partial and complete responses (**Table 8**) will be compared to the stopping rule described in Table 10 by the UPCI Multi-Center DSMC after every three patients become evaluable for response. For each number of evaluable patients in the left-hand columns, if the number of complete responses is less than or equal to the number in the right-hand column, the trial will be halted for lack of efficacy. The trial is derived from a Bayesian beta-binomial model with the prior determined by the number of complete responses observed in the high-dose arm of the randomized trial described in Section 2.3 (8/95). The values correspond to P (partial and complete response rate < 0.10)
> 0.8.

# Patients	#Responses
18	0
21	1
24	1
27	1
30	2
33	2
36	2

*Table 8.*Monitoring rule for complete response. For the given number of patients in Column 1, if the number of partial or complete responses is less than or equal to the number in Column 2, the trial is halted for lack of efficacy.

13.4 Justification of Design

The primary objective of this study is to estimate the clinical response rate of patients with metastatic RCC treated with IL-2 combined with hydroxychloroquine at 600mg/d. Assuming no TLTs occur and the trial is not closed early for toxicity or inadequate response (Section 13.3.2), there will be 39 patients available for this objective. A 95% exact binomial confidence interval on 5 responses out of 39 patients (expected if the addition of HCQ to IL-2 doubles the historic proportions of CR ~ 8%) would have width equal to 0.27 (0.07,0.34). For the secondary endpoints of OS and PFS, if patients are recruited over 15 months, observed for 15 additional months, and the true median survival is 16 months, then the expected width of the 95% confidence interval for median survival is 12.8 months.

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APPENDIX A

Procedure for Obtaining a Urine Protein:Creatinine Ratio (Or alternatively calculate from the serum creatinine based on gender and age)

1) Obtain at least 4 ml of a random urine sample (does not have to be a 24 hour urine).
2) Determine protein concentration (mg/dL).
3) Determine creatinine concentration (mg/dL).
4) Divide #2 by #3 above: urine protein / creatinine ratio = protein concentration (mg /dL) / creatinine concentration (mg /dL).

The UPC directly correlates with the amount of protein excreted in the urine per 24 hrs (i.e. a UPC of 1 should be equivalent to 1g protein in a 24hr urine collection).

Protein and creatinine concentrations should be available on standard reports of urinalyses, not dipsticks. If protein and creatinine concentrations are not routinely reported at an institution, their measurements and reports may need to be requested

APPENDIX B - Schedule of Events

Study Visit	Screening		Study start	Each Course (1-3)								End of Study Visit [q]	Follow Up [r]
	Days -41 to -14	Days -27 to -14	Day -14	Day 1	Days 7-14	Day 15	Day 29	Day 43	Day 57	Day 71	Day 84		
History/Physical exam		X	X	X		X	X		X			X	X
Concomitant medications	X	X	X	X		X	X		X			X	X
Adverse events	X	X	X----------								----X	X	X
Radiologic exams	X[a]								X[b]		X		X[b]
Pulmonary Function Testing	X[c]												
Cardiac Stress Test	X[d]												
12-lead EKG	X												
ECHO or MUGA for LVEF	X[e]										X[e]		
Screening labs		X[f]											
Urine protein:creatinine ratio		X											
Urinalysis [g]		X		X		X							
Patient Registration		X											
Treatment labs			X[h]	X[i]	X[j]	X[i]	X[h]	X[h]	X[h]	X[h]			X[h, k]
IL-2 [l]				X[l]		X[l]							
Hydroxychloroquine [m]			X----------										----X[k]
Research blood draws [n]			X	X		X[n]							
Pathology submission						X[o]							
Telephone contact for toxicity assessment					X								
Subject Drug Administration Diary for HCQ Tablets			X	X			X		X			X	X[k]

a. Within 4 weeks prior to day –14 patients will have CT scan of chest/abdomen/pelvis and brain MRI. If clinically indicated a bone scan should be done.

b. After week 8 (+/- 1 week) and week 12 (+/- 1 week) of each course patients will have CT of chest/abdomen/pelvis. If clinically indicated repeat bone scan and brain MRI. After the final course (week 36), patients will have CT scan evaluation every 12 weeks for up to one year or until tumor progression (whichever occurs first). For patients who have not shown progression at the end of one year, response will be captured during follow-up survival via telephone contact and medical record review. If additional scans are performed for clinical reasons, the study schedule may be altered to perform the requisite scans at a more acceptable time point. The change in timing of study scans must be approved by the local PI and documented.

c. All patients over 50 years of age or those with significant pulmonary or smoking history.

d. All patients over 40 years of age or with significant cardiac history.
e. ECHO or MUGA will be performed at baseline. ECHO will be performed prior to start of each new course only if clinically indicated (section 10.3.3).
f. Screening labs to include CBC w/differential, CMP (creatinine, BUN, albumin, total protein, glucose, electrolytes [Na, K, Cl, HCO3], liver function tests [total bilirubin, ALP, AST, ALT]), direct bilirubin, calcium, phosphorus, LDH, TSH, PT, PTT, HIV, Hep B and C, Pregnancy test for women of childbearing potential.
g. Proteinuria level must be checked prior to each cycle of IL-2.
h. Outpatient labs to include CBC w/differential, CMP (creatinine, BUN, albumin, total protein, glucose, liver function tests [total bilirubin, ALP, AST, ALT], electrolytes [Na, K, Cl, HCO3]), LDH, CPK, amylase, and lipase.
i. Inpatient labs to include CBC w/differential, CMP (creatinine, BUN, albumin, total protein, glucose, liver function tests [total bilirubin, ALP, AST, ALT], electrolytes [Na, K, Cl, HCO3]), direct bilirubin, calcium, magnesium, phosphorus, LDH, CPK, amylase, and lipase.
j. Outpatient labs to include CBC w/differential, CMP (creatinine, BUN, albumin, total protein, glucose, liver function tests [total bilirubin, ALP, AST, ALT], electrolytes [Na, K, Cl, HCO3]), amylase, and lipase
k. Patients with no evidence of progression by RECIST 1.1 criteria may receive continued treatment with hydroxychloroquine through one year of initiation of HCQ treatment (Day -14) until disease progression.
l. IL-2 every 8 hours on days 1-5 (or 6) and 15-19 (or 20) (maximum 14 doses per 5 day cycle). Dependent upon when Dose 1 is administered on Day 1/Day 15, Dose 14 could require hospitalization on Day 6/Day 20.
m. HCQ daily dosing starting on Day -14 (minimum of 14 days, plus a window of + 7 days if needed)
n. See Table 6 for sampling schedule. Cycle 2 draws will only be done in Course 1.
o. Blocks or slides will be sent within 4 weeks after enrollment.
p. Subjects will be evaluated with physical examination ~30 days (28-42 days) after being removed from study for any reason.
q. After the final course of treatment, follow-up procedures will be performed every 3 months (+/- 2 weeks) for up to one year or until disease progression (whichever occurs first). Follow-up procedures will include CT scan, routine laboratory tests, and physical examination. Subjects will then be followed for survival by telephone contact and medical record review every 3 months for one year following the time of disease progression or after one year off of treatment, then every 6 months for the next two years, then annually until death. A window of +/- 2 weeks is permitted.

If a subject's treatment and/or testing days need to be rescheduled due to the subject's inability to comply with the study calendar (i.e., hospitalizations, business and vacation travel plans, illness, transportation issues, holidays, family emergencies, etc.), a window of +/- one week is available for rescheduling of treatment and procedures per the discretion of the treating physician investigator, and as discussed with the principal investigator.

Appendix C

Blood Draw Instructions for Research Labs for Protocol IL-2HQ

A Phase II Study of Hydroxychloroquine and Aldesleukin in Patients with Advanced RCC

Comments	Treatment Day	Timepoint	Tube(s) to draw	Storage condition
Hydroxychloroquine is oral	Day -14	Pre-dose of Hydroxychloroquine	2 lge (10ml) Red top 10 lge (10ml) sod. Hep. Green top 1 PAXgene RNA blood tube (2.5ml)	1 on ice or in frig., 1 ambient 10 tubes at ambient temp. on their side 1 tube on ice or in frig
	Course 1 Cycle 1 Day 1 and 2	**Please draw blood before pre-treatment ancillary medication is given** Pre-dose of IL-2 (post-dose PO HCQ)	2 lge (10ml) Red top 10 lge (10ml) sod. Hep. Green top 1 PAXgene RNA blood tube (2.5ml)	1 on ice or in frig., 1 ambient 10 tubes at ambient temp. on their side 1 tube on ice or in frig
		Just prior to Dose 4 of IL-2 (+ / - 1 dose)	1 lge (10ml) Red top	On ice or in frig.
	Course 1 Cycle 1 Day 15 and 16	Pre-dose of Hydroxychloroquine	2 lge (10ml) Green top 1 lge (10ml) Red top	Ambient temp. on their side On ice or in frig.
		just prior to Dose 4 of IL-2 (+ / - 1 dose)	1 lge (10ml) Red top	On ice or in frig.
	Course 1 Between Days 60-80	During clinic visit	2 lge (10ml) Red top 10 lge (10ml) sod. Hep. Green top 1 PAXgene RNA blood tube (2.5ml)	1 on ice or in frig., 1 ambient 10 tubes at ambient temp. on their side 1 tube on ice or in frig
	Course 2 Cycle 1 Day 1	Pre-dose of IL-2 (post-dose PO HCQ)	2 lge (10ml) Green top 1 lge (10ml) Red top	Ambient temp. on their side On ice or in frig.
	Course 3 Cycle 1 Day 1	Pre-dose of IL-2 (post-dose PO HCQ)	2 lge (10ml) Green top 1 lge (10ml) Red top	Ambient temp. on their side On ice or in frig.

APPENDIX D

Processing Specimens for RCC Study (hydroxychloroquine / Aldesleukin for RCC)

1 Large Red Top Tube(s) for Serum (Kept on ice): Please carry out in laminar flow hood to keep serum sterile.

- After blood is drawn, invert tube several times to mix well. Send to UPCI IMCPL overnight.
- IMCPL Centrifuge for 10 minutes at 900g to 1000g to separate out serum.
- Aliquot serum into prelabeled sterile freeze vials at approximately 0.5 to 1.0 ml per vial (4 vials maximum).
- Fill in vial labels with patient ID# and date. (Please be careful to use appropriate lettered vials)
- Fill in Inventory/Shipping Log for W0454
- Store vials at -70 to -80°C until ready to ship.

1 Large Green Top Tube for Plasma (Kept on ice): UPCI/IMCPL will carry out in laminar flow hood to keep plasma sterile.

- After blood is drawn, invert tube several times to mix well.
- Centrifuge for 10 minutes at 900g to 1000g to separate out plasma.
- Aliquot plasma into prelabeled sterile freeze vials at approximately 0.5 ro 1.0 ml per vial (4 vials maximum).
- Fill in vial labels with patient ID# and date. (Please be careful to use appropriate lettered vials)
- Fill in Inventory/Shipping Log for IL-2HQ
- Store vials at -70 to -80°C until ready to ship.

1 10cc Red Top Tube for Serum (kept at ambient temp.) AND
10 Large Sodium Heparin Green Top Tubes for PBMCs. (Kept at ambient temp.):

Batch specimens from one patient and ship on dry ice along with a copy of the Inventory / Shipping Log, for next day morning delivery by FedEx on a Monday-Thursday to:

Please call or e-mail to alert us of shipment arrival, including tracking number.

UPCI – IMCPL
5117 Centre Avenue
1.27 Hillman Cancer Center
Pittsburgh, PA 15213
Attn: Specimen processor
Phone 412-623-1418

APPENDIX E

Inventory / Shipping Log for IL-2HQ Research Blood Specimens

IL-2HQ: A Phase II Study of hydroxychloroquine and Aldesleukin in Patients with Metastatic RCC

	Patient Study ID# _____ **Institution** _____				**Date sent to UPCI**
Day -14	**"A" Pre-dose hydroxychloroquine** 10 green tops 2 red tops 1 PAXgene RNA blood	Date	Time	am/pm	
Day 1 - 2 **Cycle 1** **Course 1**	**"B" Pre-dose IL-2 (Post-dose PO HCQ)** 10 green tops 2 red tops 1 PAXgene RNA blood	Date	Time	am/pm	
	"C" Prior to IL-2 Dose 4 (+/- 1 Dose) 1 red top	Date	Time	am/pm	
Day 15-16 **Cycle 2** **Course 1**	**"D" Pre-dose hydroxychloroquine** 2 green tops 1 red top	Date	Time	am/pm	
	"E" Prior to IL-2 Dose 4 (+/- 1 Dose) 1 red top	Date	Time	am/pm	
Between Days 60-80 **Course 1**	**"F" During clinic visit** 10 green tops 2 red tops 1 PAXgene RNA blood	Date	Time	am/pm	
Day 1 **Cycle 1** **Course 2**	**"G" Pre-dose IL-2 (Post-dose PO HCQ)** 2 green tops 1 red top	Date	Time	am/pm	
Day 1 **Cycle 1** **Course 3**	**"H" Pre-dose IL-2 (Post-dose PO HCQ)** 2 green tops 1 red top	Date	Time	am/pm	
Green top tube (8-10 mL/tube), Red top tube (8-10 mL/tube), PAXgene tube (2.5 mL/tube)					

APPENDIX F

Subject Drug Administration Diary for Hydroxychloroquine (HCQ) Tablets

SUBJECT DRUG ADMINISTRATION DIARY FOR HYDROXYCHLOROQUINE TABLETS	
Protocol ID: UPCI 11-080	**Study Drug: Hydroxychloroquine (HCQ)**
Subject Identification #:	**Study Drug Start Date:**

INSTRUCTIONS TO THE PATIENT:

1. You will take a dose of hydroxychloroquine (HCQ)_____mg daily starting on day –14. You will take:_____200 mg tablets in the morning and_____200 mg tablets in the evening every day while you are participating in this study.
2. You will start taking HCQ on day –14. You will take HCQ daily as directed.
3. For every day you take HCQ, record the date, the number of capsules you took, and the time of day you took each capsule.
4. You should take your HCQ tablets at about the same time each day.
5. Swallow the tablets whole. Do not chew the tablets.
6. Missed and vomited doses should not be made up.
7. If you have any comments or notice any side effects, please record them in the "Comments" column.
8. Please bring this form with you when you return for your doctor's appointments.
9. If you make a mistake on the form, please cross out your mistake with a single line through it. Then use the next day's space to correct the entry. Then cross out each of the subsequent numbers and write in the correct number.
10. **DO NOT SHARE THIS MEDICATION WITH ANYONE.**

SUBJECT DRUG ADMINISTRATION DIARY FOR HYDROXYCHLOROQUINE TABLETS							
Protocol ID: UPCI 11-080				**Study Drug: Hydroxychloroquine (HCQ)**			
Subject Identification #:				**Study Drug Start Date:**			
Course #: N/A (Day –14 to Day –1)				**Course Start Date:**			
Study Day	**Date**	**AM DOSE ____200 mg tablet(s)**		**PM DOSE ____200 mg tablet(s)**		**Comments**	**Missed Doses**
		# pills taken	**Time taken**	**# pills taken**	**Time taken**		
–14							
–13							
–12							
–11							
–10							
–9							
–8							
–7							
–6							
–5							
–4							
–3							
–2							
–1							

SUBJECT DRUG ADMINISTRATION DIARY FOR HYDROXYCHLOROQUINE TABLETS							
Protocol ID: UPCI 11-080				**Study Drug: Hydroxychloroquine (HCQ)**			
Subject Identification #:				**Study Drug Start Date:**			
Course #:				**Course Start Date:**			
Study Day	**Date**	**AM DOSE ____200 mg tablet(s)**		**PM DOSE ____200 mg tablet(s)**		**Comments**	**Missed Doses**
		# pills taken	**Time taken**	**# pills taken**	**Time taken**		
1							
2							
3							
4							
5							
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SUBJECT DRUG ADMINISTRATION DIARY FOR HYDROXYCHLOROQUINE TABLETS							
Protocol ID: UPCI 11-080				**Study Drug: Hydroxychloroquine (HCQ)**			
Subject Identification #:				**Study Drug Start Date:**			
Course #:				**Course Start Date:**			
Study Day	**Date**	**AM DOSE ____200 mg tablet(s)**		**PM DOSE ____200 mg tablet(s)**		**Comments**	**Missed Doses**
		# pills taken	**Time taken**	**# pills taken**	**Time taken**		
29							
30							
31							
32							
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SUBJECT DRUG ADMINISTRATION DIARY FOR HYDROXYCHLOROQUINE TABLETS							
Protocol ID: UPCI 11-080				**Study Drug: Hydroxychloroquine (HCQ)**			
Subject Identification #:				**Study Drug Start Date:**			
Course #:				**Course Start Date:**			
Study Day	**Date**	**AM DOSE ____200 mg tablet(s)**		**PM DOSE ____200 mg tablet(s)**		**Comments**	**Missed Doses**
		# pills taken	**Time taken**	**# pills taken**	**Time taken**		
57							
58							
59							
60							
61							
62							
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SUBJECT DRUG ADMINISTRATION DIARY FOR HYDROXYCHLOROQUINE TABLETS							
Protocol ID: UPCI 11-080				**Study Drug: Hydroxychloroquine (HCQ)**			
Subject Identification #:				**Study Drug Start Date:**			
Course #: N/A (Day 267 to Day 351)				**Course Start Date: N/A**			
Study Day	**Date**	**AM DOSE _____200 mg tablet(s)**		**PM DOSE _____200 mg tablet(s)**		**Comments**	**Missed Doses**
		# pills taken	**Time taken**	**# pills taken**	**Time taken**		
267							
268							
269							
270							
271							
272							
273							
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275							
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SUBJECT DRUG ADMINISTRATION DIARY FOR HYDROXYCHLOROQUINE TABLETS							
Protocol ID: UPCI 11-080				**Study Drug: Hydroxychloroquine (HCQ)**			
Subject Identification #:				**Study Drug Start Date:**			
Course #: N/A (Day 267 to Day 351)				**Course Start Date: N/A**			
Study Day	**Date**	**AM DOSE _____200 mg tablet(s)**		**PM DOSE _____200 mg tablet(s)**		**Comments**	**Missed Doses**
		# pills taken	**Time taken**	**# pills taken**	**Time taken**		
295							
296							
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SUBJECT DRUG ADMINISTRATION DIARY FOR HYDROXYCHLOROQUINE TABLETS							
Protocol ID: UPCI 11-080				**Study Drug: Hydroxychloroquine (HCQ)**			
Subject Identification #:				**Study Drug Start Date:**			
Course #: N/A (Day 267 to Day 351)				**Course Start Date: N/A**			
Study Day	**Date**	**AM DOSE _____200 mg tablet(s)**		**PM DOSE _____200 mg tablet(s)**		**Comments**	**Missed Doses**
		# pills taken	**Time taken**	**# pills taken**	**Time taken**		
323							
324							
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SUBJECT DRUG ADMINISTRATION DIARY FOR HYDROXYCHLOROQUINE TABLETS							
Protocol ID: UPCI 11-080				**Study Drug: Hydroxychloroquine (HCQ)**			
Subject Identification #:				**Study Drug Start Date:**			
Course #: N/A				**Course Start Date: N/A**			
Study Day	**Date**	**AM DOSE _____200 mg tablet(s)**		**PM DOSE _____200 mg tablet(s)**		**Comments**	**Missed Doses**
		# pills taken	**Time taken**	**# pills taken**	**Time taken**		
