Research Article

Chrono-Immunotherapy with Interleukin-2 for Treatment of Metastatic Renal Cell Carcinoma: A Phase I-II Study

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Abstract

Background: IL-2 represents yet an alternative to targeted therapy for metastatic renal cancer (mRCC) **treatment.** Prior studies have suggested high dose bolus as the preferred administration modality. However, its use is limited by toxicity and the search for an alternative modality is justified. In order to reduce toxicity we evaluated feasibility, dose limiting toxicity (DLT) and activity of IL-2 chrono-infusion in mRCC.

Methods: Patients (pts) with mRCC were treated with IL-2 which was administered with an escalated dose in 8 hour chrono-infusion (peak 1am, 9am, and 5pm) from 2 MUI/m^2 according to Fibonacci scale.

Results: 22 pts were enrolled. 3 pts entered each Level I to V and 7 entered VI. Grade 3-4 toxicities were reported only in 4 cases (2 hypotension and renal, 1 diarrhoea) on the VI Level. DLT was 18.6 MUI/m2. Overall response rate was 14% (23% in a good-intermediate risk group). Median PFS and OS were 4.5 and 14.5 months respectively. In an univariate analysis dose level, PS, gender, cD3, cD4, cD8 counts were statistically significant for OS.

Conclusion: IL-2 chrono-infusion appears safe in a standard care unit, moderately toxic and active in mRCC.

ABBREVIATIONS

IL-2:Interleukin-2; Ifnα: Interferon Alfa:; Mrcc: Metastatic Renal Cell Carcinoma; CTLA-4: Cytotoxic T Lymphocyte-Associated Antigen-4; LAK: Lymphokine-Activated Killer:; FU: Fluorouracil; TNF-Alpha: Tumor Necrosis Factor Alfa; IL-1 Beta Interleukin-1 Beta; IL-6: Interleukin-6:; ECOG PS: ECOG Performance Status; CR: Complete Remission; PR: Partial Remission; SD: Stable Disease; PD: Progression Of Disease; DLT: Dose Limiting Toxicity; RR: Response Rate; PFS Progression Free Survival; OS Overall Survival; NCI (CTCAE: Common Terminology Criteria Version 3.0 Grading System; DCR: Disease Control Rate; AM: Ante-Meridian; PM: Post-Meridian; MSKCC: Memorial-Sloan-Kettering-Cancer Center; CCF: Cleveland Clinical Fundation; T-Reg: T Cell Regulator

INTRODUCTION

Renal cell carcinoma (RCC) represents 5.2% and 2.8% of all cancers in men and women respectively [1].

Systemic cytokines IL-2 or interferon-α (IFN-α) long-time represented the standard of care for metastatic RCC (mRCC) [2]. In the cytokines era median overall survival for mRCC patients was around 1 year and only 10% of such patients survived past 5 years

[3]. The last few years have seen a steep rise in pharmacological research in mRCC, mainly due to the introduction of novel agents targeting angiogenesis and signal transduction pathways, such as tyrosine kinase and rapamycin inhibitors [4-9], which have significantly improved patient outcomes. The availability of new agents and consequently the sequential use of targeted therapies can result in a progression-free survival of up to 27 months, and an overall survival of 40 months [10], that represents an impressive progress. At present, drugs such as Sunitinib [4], Pazopanib [8], Bevacizumab [9], Sorafenib [5], Everolimus [6], Temsirolimus [7], and recently Axitinib [11], are considered standard treatments for RCC. Despite this, treatment with targeted agents rarely achieves complete responses and most patients (pts) ultimately develop resistance to therapy [2], so a definitive cure is not provided. It is well known that some pts treated with cytokines, especially with high-dose IL-2 experience durable complete responses up to 2 years [12]. Recently, in patients with other tumour types, an impressive comeback of the use of immunotherapeutic agents has been associated with substantial survival improvements. In a phase III trial of advanced or metastatic melanoma, Ipilimumab, an antibody against cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) [13], showed remarkable improvement in overall survival. Similarly, in a placebo-controlled phase III trial of patients with castration-resistant prostate cancer, sipuleucel-T, an autologous cellular vaccine, improved median survival [14]. The proof-of-principle that immunotherapy has the potential to improve outcomes in patients with advanced cancer and the fact that targeted agents have limited capacity to produce durable complete responses [15], suggests continuing the investigation of the use of immunotherapy in mRCC. Either new immunotherapy drugs or different strategies of the use of older drugs are rationale options. The main concern in using immunotherapy has always been represented by the significant toxicity. At present biomarkers of response have yet to be identified to identify those patients who could respond and those who should avoid exposure to toxicity. Thus, despite the fact that high-dose IL-2 is still a valid therapeutic option, there is a general agreement that its use should be restricted to small subgroups of patients, namely young and fit pts only, who can probably better tolerate such therapy [2,12].

IFN alfa has shown higher response and survival compared to other drugs [16-19] and when added to nephrectomy in a randomized trial [20]. More recently, it resulted inferior to sunitinib[4], Temsirolimus[7] or to its combination with Bevacizumab [8] in pivotal trials.

IL-2 is a pleiotropic protein produced by activated T lymphocytes and represents the most important growth factor for lymphokine-activated killer (LAK), T-helper cells, eosinophils and natural Killer (NK) cells [21-24]. In a US trial, high-dose IL-2 intravenous (IV) regimen (i.e. 600 to 720,000 IU / kg bolus t.i.d for 5 days/2 weeks), determined a 16% response rate (RR) and 7-9% long-term complete response (CR) [3]. However, the interim analysis, comparing high-dose versus low-dose bolus versus subcutaneous (SC) administration, did not indicate a significant benefit of high-dose IL-2 except for CR [25]. In Europe, a continuous IV infusion of IL-2 (18 MUI/m² for 5 days/2 wks) was investigated, initially obtaining 50% RR in 13/26 solid tumours, 8/16 of which were RCC and melanoma [26]. A French randomized study comparing IV or SC IL-2 plus IFN versus IFN

alone found no significant difference in RR and OS in unselected patients [27]. In the Atzpodien experience, SC IL-2 and IFN combined to Fluorouracil (FU), suggested promising RR and OS [28] but these results were not confirmed [29-31]. A randomized study comparing IFN-alfa versus combination therapy with IFN-alfa, IL-2 and fluorouracil revealed no difference in toxicity, activity and survival between the two arms [32]. In an attempt to modulate IL-2-related activity and toxicity, chronomodulation delivey has been investigated. Chronomodulation delivers drugs in a circadian rhythm, seeking to maximize dose-intensity and subsequent outcomes, while also attempting to minimize toxicity.

Few studies on more than 30 anticancer drugs analyzed different administration times. It is impressive to note that the difference in efficacy and incidence of adverse events for the same dose of the drugs could be even twofold when given at different times during the day or at night [33]. These observations suggested the study of chronomodulation in chemotherapy regimens [34] and several agents were found suitable for chrono-therapy [35]. Chronomodulation is based on the interactions between the drugs and the human body that are modified by the circadian rhythm and can impact on cell growth and proliferation. The mechanisms of such complex nictemeral interaction with the immune and hormonal system and their environment are yet unclear. The master pace-maker of the physiological circadian rhythm is represented by the suprachiasmatic nucleus (SN) which is located in the anterior hypothalamus. SN is involved in homeostatic, behavioural and neuroendocrine functions and also in potentially tumour-modulating effects, because it rules processes with relevant impact for the neoplastic progression [17] such as cell-cycle gating, DNA repair, apoptosis, angiogenesis and immune function. Thus its dysregulation may be an important promoter and inducer of cancer [37]. A cross talking between paraventricular hypothalamus and SN is influenced by blood-borne signals, since pro-inflammatory cytokines determine depression or sleep disorders, moreover a relationship between acute/chronic stress, endogenous/therapeutic glucorticoid concentration and immune activation is described [38-45]. The negative impact of circadian variations and stress, appears greater with TNF-alpha and IL-1 beta than IL-6 [46], explaining the significant reduction of IL-1 after the first phase of sleep [43].

The synchronization of endogenous IL-2 by IL-1 [47] may play an important role in an immunogenic tumour model such as RCC. As chrono-infusion allows the administration of concentrated doses of drugs and its modulation over time, this led us to study the use of chrono-modulated IL-2 and to evaluate the possible benefits of chrono-infusion by its interactions with immune and hormonal system in RCC

MATERIALS AND METHODS

Patient selection

Eligibility criteria include: age below 70 years, ECOG PS < 2, histological diagnosis of mRCC, measurable metastatic disease (NCIC criteria), adequate organs function (hemoglobin >10 g/dL white blood cells > 4000/mm3, neutrophil >1500/mm3, creatinine < 1.7 mg / dL (150 micromol / L), ALT < 2 x ULV, bilirubin <1.5 mg / dL x ULV, ECG and echocardiogram without any great abnormality and ejection fraction > 50%, written informed consent. Study was performed in accordance with the Declaration of Helsinki.

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Pts who had received previous treatment with IL-2, IFN-alpha, targeted-therapy, chemotherapy and radiotherapy more than 6 weeks before were included.

Ineligibility criteria were represented by the presence of clinically active brain metastases and seizures, recent cardiac illness (heart failure, angina, myocardial infarction, and ventricular arrhythmia), major infections, contraindications to the use of vasopressor drugs (e.g dopamine), chronic corticosteroids use, and previous history of organ transplantation.

Study design and treatment

Primary objective: safety, dose limiting toxicity (DLT).

Secondary objective: correlation between therapy and hormonal and immunological order, response rate (RR), progression free survival (PFS) and overall survival (OS).

IL-2 was diluted in 5% glucose solution enriched with 13 ml of human albumin at 20% days was administered by venous port device. The dose of chronomodulated IL-2 (Figure 1) was made according to the 6 levels of the Fibonacci scale (Table 1). The cycle was repeated every 2 weeks x 4 cycles. If tolerability was good, objective response or stabilization (WHO) were obtained, further 4 cycles were delivered every 3 weeks. Duration of response, PFS and OS were evaluated from start of IL-2 to progression, death or last visit respectively.

Toxicity was assessed according to NCI Common Terminology Criteria version 3.0 grading system (CTCAE v3.0) [48]. The definition of DLT for the IV chrono-infusion of IL-2 was the occurrence of renal toxicity (oligo-anuria), hypotension, cardiac toxicity greater than G2, sensory or motor neurological toxicity > G1, or serious intolerance that contraindicate further treatment.

Statistical analysis

The study design provides for the allocation of 1 patient in each of the three pre-ordered chronomodulation times for each level of the Fibonacci scale (Table 1). After treatment of three pts/level, if no event of DLT occurred, we proceeded to the next level. If one DLT event has occurred, it was necessary to enter 3 other patients at the same level. If no DLT events were observed, the next level was entered. In the presence of two DLT events at any level, the dose escalation was blocked.

The calculation of the number of patients required for this Phase II was made by Simon's method in two steps [49]. Whereas we wanted to obtain a 20% difference (p1-p0 = 20%) between the standard therapy (p0 = 20%) and the new therapy (p1 = 40%) and fixed the error α= 0.05 and β= 0.20 (1-beta = 0.80; power of the test), the number of patients required for the 1st step was 13. If response numbers were 0-< 3, the study would be completed. On the contrary, the study continued up to the enrolment of 43 patients. The response numbers rejecting the null hypothesis (ineffective treatment) were to observe more 12 from 43 under treatment. Differences between discrete variables were analyzed using the chi-square or Fisher exact test for expected values <5 [50].

Differences between continuous variables were analyzed using the Mann-Whitney nonparametric test (for comparison of two independent samples). The results were considered statistically significant for values of $p < 0.05$ (two-tailed test)

RESULTS AND DISCUSSION

From January 2005 to July 2009, 22 pts (Table 2) were enrolled in the study. 3 pts entered each in level I-V, 7 in level
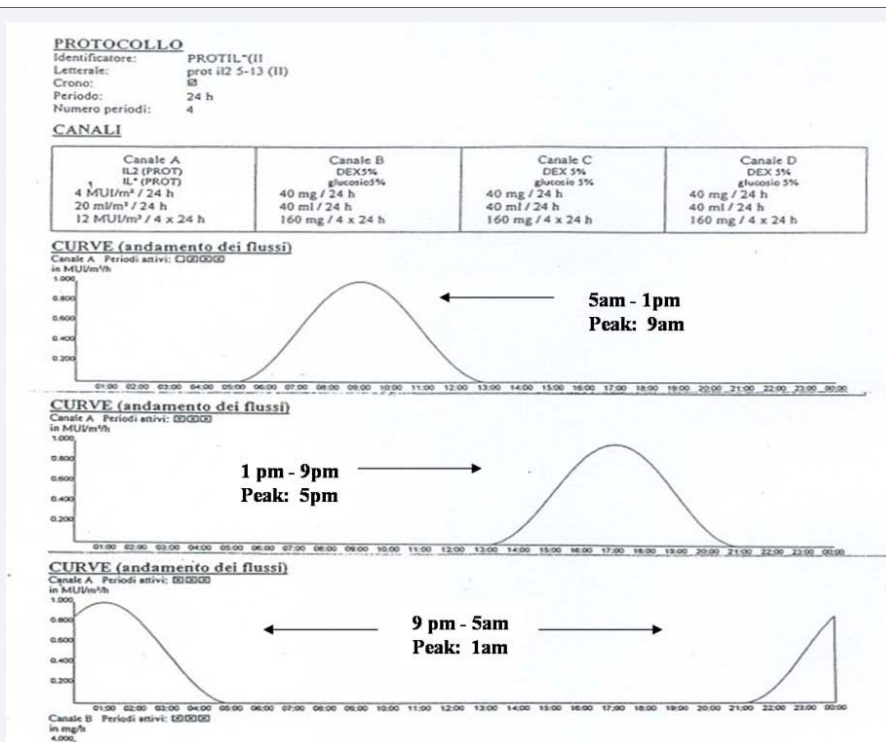

Figure 1 Three times (5am-1pm; 1pm-9pm; 9pm-5am) of chronotherapy; p: peak.

Table 1: Fibonacci scale of IL-2 dose.

Level	Time (9pm-5am) peak 1am	Time (5am-1pm) peak 9am	Time(1pm-9pm) peak 5pm	Dose (MUI/m²)	% increase
I	1	1	1	2	100
II	1	1	1	4	67
III	1	1	1	6,68	50
IV	1	1	1	10	40
V	1	1	1	14	33
VI	1	1	1	18,6	

Table 2: Patients characteristics.

Factors	N (range)
SEX M/F	16/06
MEDIAN AGE	68 (44-74)
MEDIAN PS (range)	1 (0-1)
Nefrectomy (yes:no)	20:2
Histology (CC: sarcomatoid)	20:2
METASTASES SITES lung	16
liver	4
lymphnodes	5
bone	10
soft tissue	3
other	9
N° metastatic sites 1	8
2	5
> 3	9
Motzer modified score 0-1	6
2	7
3	9
naive pts	14
pre-treated pts	8
immunotherapy	2
targeted therapy	6
chemoimmunotherapy	4

VI. 106 cycles were administered. Median relative dose intensity (MRDI) were 98.5%, 91%, 89%, 85%, 88%, 65% for Level I, II, III, IV, V, VI respectively.

All pts experienced flu-like syndrome, but only 4 pts had G3-4 toxicity (2 hypotension, 1 renal and 1 diarrhoea) on Level VI. One out of 4 of pts pre-treated with Sunitinib experienced myocardial infarction after a few months of Il-2 interruption, without significant impact on further therapy. Overall, no cardiac toxicity in terms of ejection fraction reduction was detected. DLT was 18.6 MUI /m².

Out of 22 treated pts 1 CR, 2 PR, 9 SD, 10 PD were reported. The overall response (OR) and disease control rate (DCR) were 14% and 54% respectively.

2 patients obtained mixed response (Level VI) (supraclavicular nodes CR and liver PD; gastric PR, lung and adrenal PD in 1 pt respectively). In the good-intermediate risk group, RR and DCR was 23% and 69%. Median PFS and OS (Figure 2) were 4.5 and 14.5 months respectively.

In a univariate analysis (Table 3) age (< 65 y vs >65 y), Motzer score (0-2 vs. 3) increase/reduction of cD19, cD16, HLA-DR, cD4/cD8, increase/reduction of prolactine, reduction/increase of ACTH-Cortisol were not statistically significant for OS. However a survival trend (Figure 3) was detected in the peak treatment (1:00 AM and 9:00 AM versus 5:00 PM) groups, Motzer groups, cD16 and cD19 counts, cD4/cD8, Prolactine, ACTH and cortisol. Conversely, dose levels (4-6 vs. 1-3), PS (0 vs. 1-2), gender (male vs. female) and cD3, cD4, cD8 count (gain vs. drop) were statistically significant for OS (Figures 4-9).

DISCUSSION

Before the introduction of tyrosine kinase and rapamycin, IL-2 represented the cornerstone of mRCC treatment. However, its activity is not generalized to all histologies, as the presence of more than 50% of alveolar features without granular or papillary component has a greater probability of response to IL-2 [51], but no other predictive factors are yet known. A meta-analysis from retrospective studies on high dose IL-2 demonstrated that response was the strongest predictor of OS and more than "4 years" survival rate were 62% and 11% in responders and non responders respectively [52]. The low response rate to IL-2 suggests that, as seen for other kinds of immunotherapy, impact on survival can be a delayed effect of treatment and a 19% 5-year survival after HD IL-2 was recently reported [53]. According to the MSKCC [54] and CCF prognostic criteria [55] for RCC, all variables of the Motzer model, except ECOG PS, with adjunction of prior radiotherapy, lung, liver and retroperitoneal metastases, were validated as independent negative prognostic factors [55].
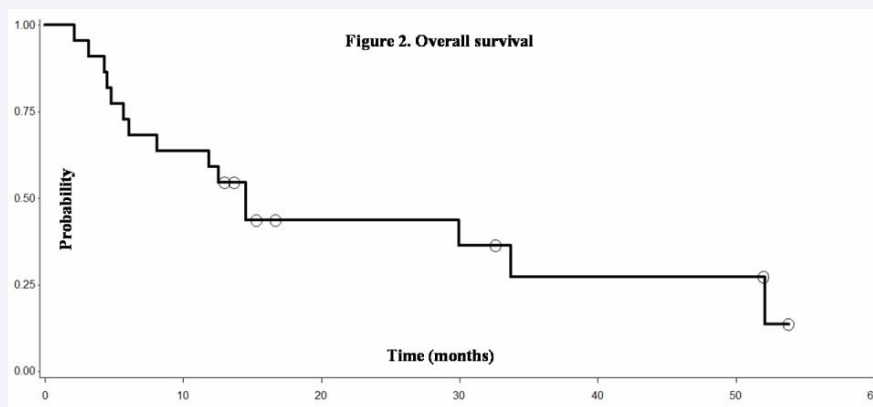
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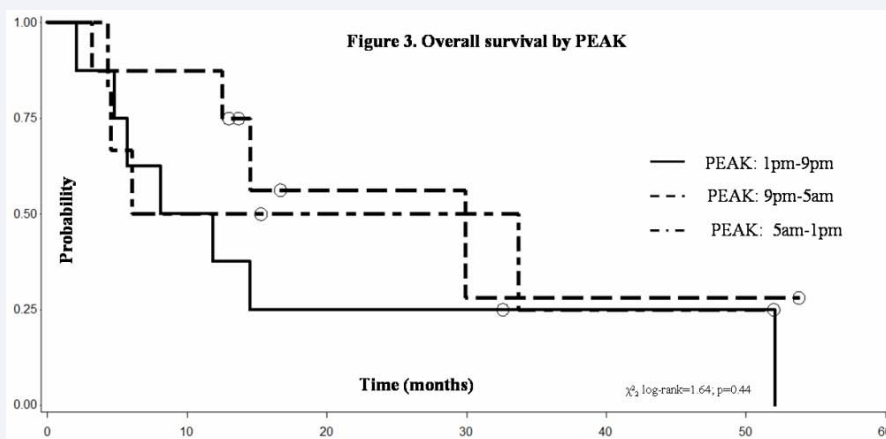
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Figure 2 Overall Survival.

Figure 3 Correlation between time (9pm-5am, 5am-1pm, 1pm-9pm), related peak (1am,9am,5pm) and Overall Survival.
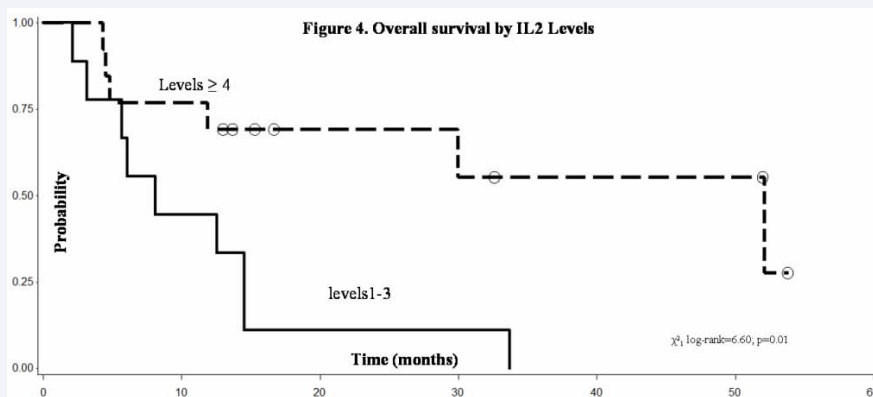

Figure 4 Correlation between Dose Level 1-3 (2-6.68 MUI/m2), 4-6 (10-18.6 MUI/m2 and Overall Survival.

Conflicting data on response to treatment and prognosis are available in patients with bone metastases [56-58] and their prognostic relevance is uncertain, also Aktins prospective model was not conclusive or helpful for the selection of potentially responsive patients [59,60].

IL-2 induced durable response in a few patients with favourable prognostic factors but, while high dose "bolus" requires an expert team in an intensive therapy unit, continuous infusion or high dose subcutaneous IL-2 can be administered in a standard care unit, or even in out-patient setting. An expert side effect management aimed at performing an adequate treatment for dose intensity and overall duration is essential to achieve results in all treatment modalities.

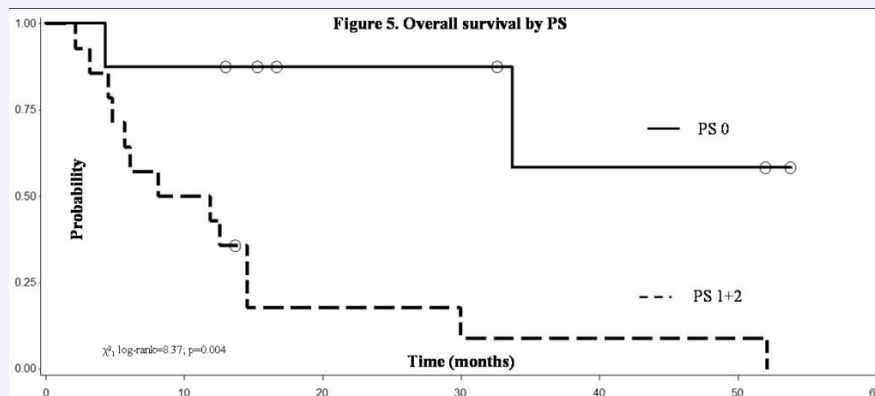This can be assumed, from the analysis of the Gore's study

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Figure 5 Correlation between ECOG PS and Overall Survivalr.
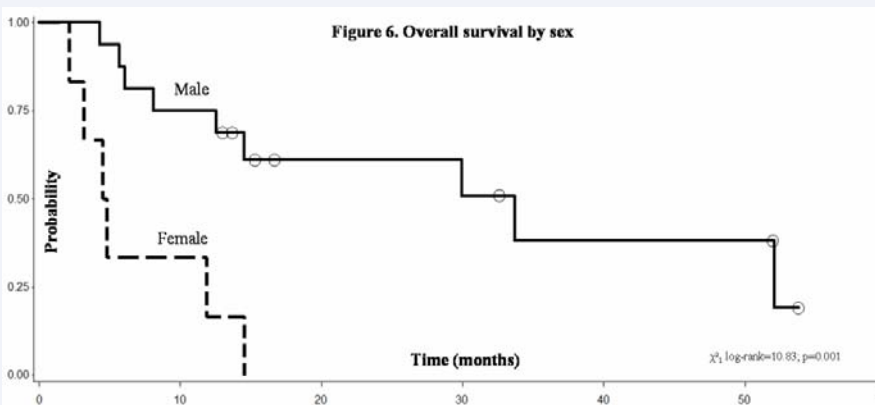

Figure 6 Correlation between gender and Overall Survival.
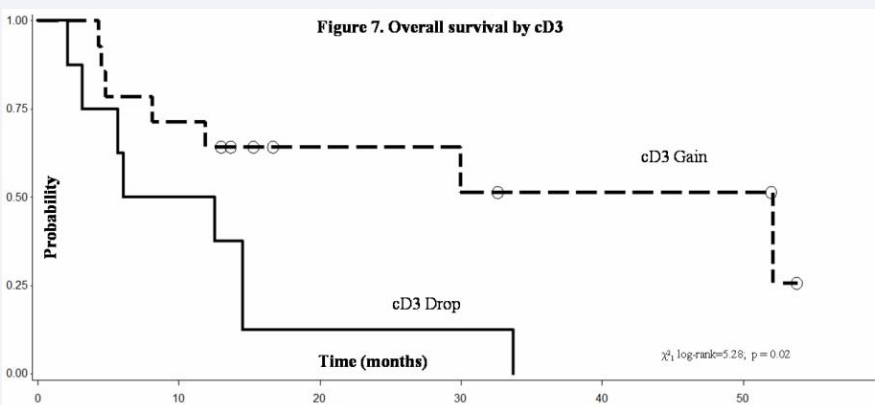

Figure 7 Correlation between cD3 outcome and Overall Survival.

[32], characterized by shortness and the low dose intensity of therapy than the Atzpodien one [28]. Nevertheless durable objective responses were reported in both studies.

Our patients treated with IL-2-IFNα -FU regimen [31] mainly underwent two cycles (and a further 2 cycles in responders). At evaluation after 13 years, 6 out of 40 treated patients were alive with disease. This emphasizes the value of Il-2 "per se" on survival with either high or low dose.

Then comes the need to use an easier administration modality and to explore new strategies to improve feasibility and results. Chronotherapy allows the Gaussian administration of drugs in a specific period of the day and it is suitable for its correlation with the hormonal and immune system.

The cyclical administration of 8 hours IL-2 offers the possibility to obtain a three times increase of dose intensity compared to the same exposure time of 24 hours c.i. It can be
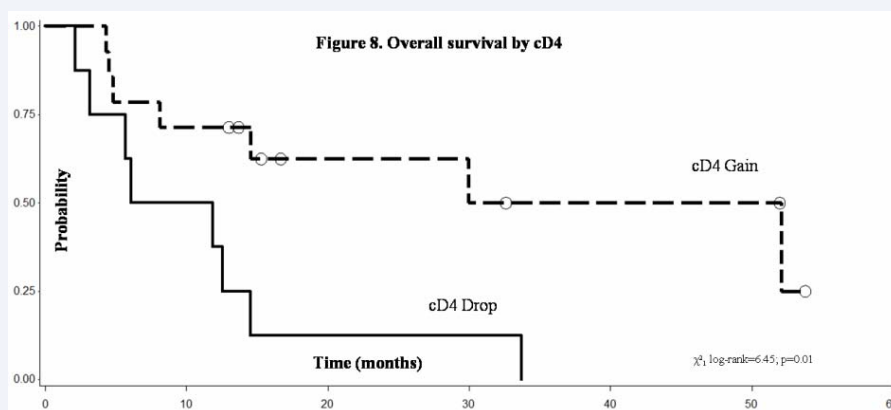
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Figure 8 Correlation between cD4 outcome and Overall Survival.
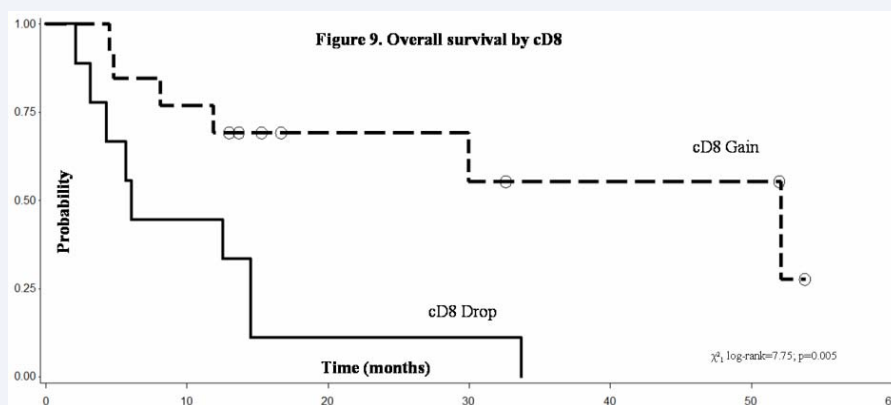

Figure 9 Correlation between cD8 outcome and Overall Survival.

likened to a "prolonged bolus". To the best of our knowledge this is the first experience regarding chronoinfusion IL-2.

The prognostic value of gender on OS is interesting, this was significant also in chrono-chemotherapy of colorectal cancer [61]. Our study also confirms the prognostic value of lymphocytosis as in m-RCC [62]. We are able to detect the impact of antemeridian peak on survival in the short-medium term of observation. It suggests the importance of treatment timing and could be explained by effects on the immune system of hormonal changes over time. Remarkably, response to IL-2 is present also in case of unfavourable disease sites such as bone and liver. In fact a durable response was obtained in a patient with bone metastases pre-treated with Sunitinib and in a therapy-naive patient with liver, bone and lung metastases. This suggests the presence of immunological changes after Sunitinib. In fact an increase of lymphoid proliferation, Interferon-γ, IL-4 producing T-cells is known, whereas T-reg and CD33+/HLA-DR- and CD15+/CD14- Myeloid-Derived Suppressor Cells (MDSC) were reduced [63,64]. Also these results confirm the value of HD IL-2 on improving outcome in patients with negative prognostic factors [57].

In our limited cohort of patients pre-treated with TK inhibitors, treatment has been feasible and less toxic than in Cho experience [65].

CONCLUSION

From this study we found that IL-2 chronoinfusion therapy showed moderate toxicity, lower than standard infusion modality, feasibility in a standard care unit and activity in unselected patients with m-RCC; DLT was reached at level 6. MTD was placed at level V. The study continues as phase II.

In the light of new knowledges about the action mechanism of new immunotherapeutic agents and the clinical improvement in solid tumors, IL-2 could still play a role in its sequential use in mRCC

ACKNOWLEDGEMENTS

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