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## Case Report

# Cyclophosphamide, Fluorouracil and subcutaneous Interleukin-2 in the treatment of advanced GIST: a case report

Giovanni Lo Re<sup>1\*</sup>, Francesco Lo Re<sup>2</sup>, Paolo Doretto<sup>3</sup>, Alessandro Del Conte<sup>1</sup>, Wally Marus<sup>4</sup>, Sandro Sulfaro<sup>4</sup>, Piero Brosolo<sup>5</sup> and Paolo Ubiali<sup>6</sup>

<sup>1</sup>CRO Aviano Medical Oncology and immunerelated tumors, Italy

<sup>2</sup>Study University, Milan, Italy

<sup>3</sup>Clinical Patholgy, AAS5 Pordenonese, Pordenone, Italy

<sup>4</sup>Pathology Unit, AAS5 Pordenonese, Pordenone

<sup>5</sup>Gastroenterology Unit, AAS5 Pordenonese, Pordenone

<sup>6</sup>Surgery Unit, AAS5 Pordenonese, Pordenone

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## ABSTRACT

A male 68 years old patient was admitted to surgical ward for hemorrhagic shock. After CT scan detection of 6x5 cm neoformation of first jejunal loop, he was submitted to segmental resection and pathological diagnosis was gastrointestinal stromal tumor. The patient was defined as high-risk according to Takahashi criteria, but refused Imatinib adjuvant therapy. After 15 months of disease-free interval, he developed bilobar liver metastases. After treatment with Imatinib 400 mg he reported G3 hepatotoxicity resolved with temporary suspension, he continue low dose with stable disease. After liver progression, he resumed Imatinib full dose with disease stabilization for 9 months. After liver progression, second line Sunitinib 37,5 mg/day was started for four months with stable disease. After further liver and lymph node mediastinal progression he was treated for four months with Regorafenib with disease stabilization. Patient developed slow but inexorable progression of liver disease with severe abdominal pain resistant to opioid and was treated with authorized compassionate program comprising Cyclophosphamide 300 mg/sqm and Fluorouracil 500 mg/sqm on day 1 intravenously followed by Interleukin-2 4.5 MUI subcutaneously on days 3–6 and 17–20 every four weeks. After three cycles the patients obtained a relevant subjective improvement with partial response on mediastinal lymph node and liver stabilization. A substantial increase on neutrophil, lymphocytes, monocytes, platelets, T regulator cells count, and a decrease on platelets/lymphocytes, CD8/T regulator cells ratio, CD8, NK count and C-reactive protein value were observed after treatment compared to basal value. The toxicity was mild represented by fever G1, flue-like-syndrome G1 during the treatment. After four cycle of chemo-immunotherapy, the patient demonstrated progression of disease and died five months after treatment. Noteworthy is the temporal disease control with significant symptomatic improvement achieved for the first time with this chemo-immunotherapeutic combination in a patient with very advanced pretreated GIST.

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## Introduction

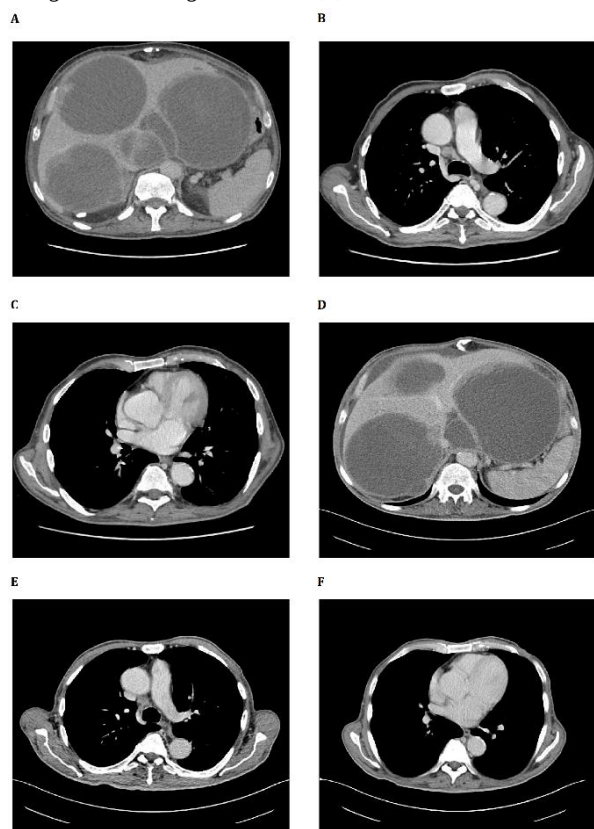
The growing interest resulting from the evidence that immune suppression plays an important role in the progression of disease in

tumors has aroused much interest in the treatment of resistant tumors. The description of this case of heavily pre-treated GIST pays attention to a combined treatment that confirms the possibility of translating into practice the theoretical assumptions underlying immunosuppression

\*Correspondence to: Giovanni Lo Re MD, CRO Aviano Medical Oncology and immunerelated tumors, Italy; Tel: 00390434399788; 00390434399187; E-mail: [giovanni.lore@cro.it](mailto:giovanni.lore@cro.it)

## Case Report

A male 68 years old patient on December 2010 was admitted to the surgical ward for hemorrhagic shock by neofunction bleeding of the first jejunal loop. The patient underwent esophagogastroduodenoscopy and rectosigmoidoscopy which were negative for neoplastic disease. The CT scan showed 5 cm round oval formation contained in the adipose tissue of proximal jejunal loop. Enhancement of the lesion wall and of the intestinal lumen contiguous to the mass, slightly dissociability from adjacent intestinal loops and no widespread metastatic lesions were detected. On December 2010 the patient was submitted to segmental resection of bleeding of 6x5 cm neofunction of the first jejunal loop. The histopathological specimen showed stromal tumor (CD117 + of membrane and paranuclear cytoplasmic, CD34 + zonal; Caldesmone-, Desmina-, S100 -) infiltrating intestinal muscle tunic, mitotic index of 15/10 HPF 40 x (Ki 67 15-20%), zonal areas of necrosis and hemorrhage. Remaining visceral wall and resection margins were free of neoplastic infiltration. Therefore, for small bowel invasion the patient was defined as high risk according to Takahashi [1].



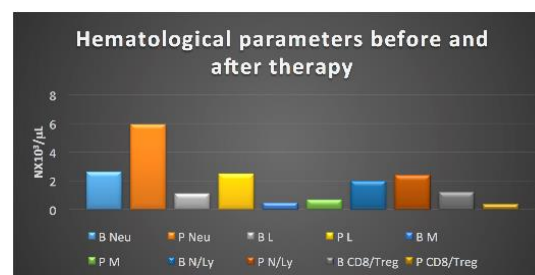
**Figure 1A:** Increased in number and size the hypodense lesions, the greater to the II segment of 16 cm. The portal vein appears focally compressed by localized I segment formation of 7.5 cm). The inferior vena cava remains thinned but still open, in the retro-hepatic tract. Presence of peri-hepatic fluid effusion

**Figure 1B:** Slight increase in size of lymph node formation in the Baretty loggia (25x14 mm) and in the right hilar area (14x16 mm) (Figure 1C)

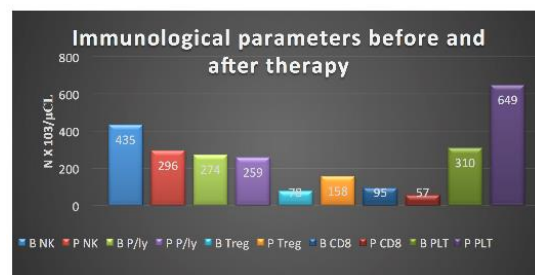
Figure 1 D) Not substantial variations in number and size but more hypodensity of the metastatic lesions. No new focal lesions in the spleen. Persistence of peri-hepatic fluid effusion

Slightly reduction of the Baretty lodge lymph node (21x13 mm) ( Figure 1 E) and the one in the right hilar area (13x7 mm) (Figure 1F)

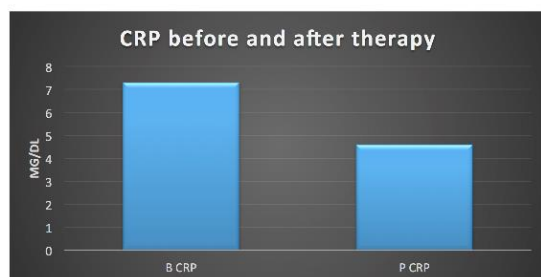
Postoperatively the patient refused Imatinib (IM) adjuvant therapy. After disease-free interval of 15 months he developed bilobar liver metastases with necrotic component in the caudate lobe (40 mm) and VII segment (35 mm). From April 2012 he was treated with IM 400 mg but he reported G3 hepatotoxicity resolved with IM suspension. Thereafter from July 2012 to May 2014 he continues IM 300 mg/day with stable disease. After liver progression, he resumed full dose IM from May 2014 to October 2015 obtaining a disease stabilization for 9 months. After liver progression, from November 2015 to March 2016 Sunitinib 37,5 mg/day was started with stable disease. After further liver and lymph node mediastinal progression he was treated from June 2016 to October 2016 with standard dose of Regorafenib with disease stabilization. Toxicities of TKI inhibitors were asthenia G1, dysgeusia, anorexia, epigastralgia, diarrhea G2. After slow but inexorable progression of liver disease (Figure 1 A) with huge, hard consistency, hepatomegaly resulting in severe abdominal pain resistant to opioid therapy and mediastinal lymph node enlargement (Figure 1 B-C), he was treated with authorized compassionate program consisting on outpatient Cyclophosphamide (Cyt) 300 mg/sqm and Fluorouracil (FU) 500 mg/sqm on day 1 intravenously followed by Interleukin-2 (IL-2) 4.5 MUI subcutaneously on days 3–6 and 17–20. The cycle was repeated on day 29. The treatment plan provided three cycles of therapy followed by further three cycles if stabilization or objective response [2]. The patients after three cycles of therapy obtained a relevant subjective improvement with liver stabilization (Figure 1 D) and partial response on mediastinal lymph node (Figure 1 E-F). A substantial increase on neutrophil, lymphocytes, monocytes, platelets, Tregs count (Figure 2 A-B), and a decrease on platelets/lymphocytes, CD8/Treg ratio, CD8, NK count (Figure 2 B) and C-reactive protein value (Figure 2 C) were observed after treatment. The toxicity was mild and was represented by fever G1, flue-like-syndrome G1 during the treatment. After four cycle of chemo-immunotherapy, the patient developed progression of disease and died five months after treatment.



**Figure 2A:** Increase of neutrophil (Neu), lymphocytes (Ly), monocytes (M), N/Ly, decrease of CD8/Ly T regulator (Treg) ratios after therapy; B: basal; P: post-therapy



**Figure 2B:** Increase of platelets (PLT), lymphocyte T regulator (Treg), decrease of PLT/Lymphocyte (Ly) (PLT/Ly), CD8, and natural killer (NK) count after therapy; B: basal; P: post-therapy



**Figure 2C:** Decrease of C-Reactive Protein (CRP) after therapy; B: basal; P: post-therapy

## Discussion

Metastatic GIST represents a chemo-resistant disease. It was considered an orphan neoplasm until 2000 when IM activity was tested, showing significant efficacy in advanced disease, especially in patients with exon 11 c-Kit mutation [3]. Furthermore, adjuvant IM have positive impact on prognosis and survival in the intermediate-high risk, according to Miettinen prognostic score [4, 5]. However, after disease progression both after IM adjuvant and palliative therapy for advanced disease, different rates of primary or secondary resistance are observed depending on the type of mutation. IM exerted immunomodulatory functions by inhibiting and/or activating specific subsets of immune cells mostly natural killer (NK) and progressive loss of MHC [6-8]. The immune system in GIST presents a dynamic picture with variations on the primary tumor and metastases and in relation to IM treatment and to c-Kit mutation that gives predictive and prognostic value. After the demonstration in the animal model of host dendritic cell (DC) revertant effect on resistance to IM through the promotion of the NK-cell-dependent antitumor effect, subsequent studies demonstrate the CD11c+B220+NK1.1+ cells increase during the combination therapy of IM plus IL-2 for the production IFN- $\gamma$  by contact with tumor cells naming these CD11c+ cells IFN-producing killer DC (IKDC) [9, 10].

Further experience of Gustave Roussy chemo-immuno-target regimen comprising oral metronomic Cyt combined to IM 400 mg/day from D-21 to D 14 before escalating dose (3, 6, 9 and 12 MIU/day) of subcutaneous IL-2 was done. The study highlighted the feasibility of the treatment, the achievement of maximum tolerated dose and the influence of IL-2 on the increase in concentration and impregnation of IM and its metabolite but without objective response [11]. Furthermore, a positive correlation between IL-2 dose and reduction of the absolute counts of B, CD4+ and CD8+ T cells, a slight increase of regulatory T cells (Tregs) rate and NK cell activation with upregulation of HLA-DR, tumor necrosis factor-related apoptosis-inducing ligand and CD56 has been demonstrated. The overexpression of HLA-DR+ natural killer cell levels were positively associated with progression-free and overall survival. Moreover, the CD4+/CD8+ T-cell ratio increase was predictor of overall survival post all treatment steps [12]. The predictive and prognostic value of CD4/CD8 ratio as of Neutrophil/Lymphocyte ratio (NLR) was reported with sunitinib in advanced GIST and are in agreement with what has been observed in other neoplastic pathologies [13-15]. Recently we have witnessed an explosion concerning the research on new immunotherapeutic agents that have come into clinical practice both in solid and hematological tumors.

However, whereas some efficacy has been found with ipilimumab alone

and in combination with nivolumab [16, 17], these results have not been confirmed by with the combination of metronomic Cyt to anti- PD1 Pembrolizumab in pretreated soft tissue sarcoma (STS) patients and GIST, characterized by low expression of PDL-1  $\geq 1\%$  on immune cells and high expression of CD163-positive macrophages with M2 phenotype, indoleamine 2,3-dioxygenase 1 and its product Kynurenine. These factors play a role in immune suppression and resistance to PD-1/PD-L1 targeting in STS and GIST [18]. In light of the uncertainty on the efficacy data of the new anti-CTL-A4 inhibitors and Anti-PD-1/anti-PDL-1 check-point agents, and the direct and indirect immunosuppression determined by Treg and MDSC, the rediscovery of the effectiveness of IL-2, assisted by immune-modulating agents could be justified. IL-2 is produced by activated T cells and increase the proliferation and activation of cytotoxic T cells, natural killer (NK) cells and monocytes [19]. IL-2 is essential for the maintenance and expansion of immunosuppressive CD4+CD25+ Tregs, in which IL-2R $\alpha\beta\gamma$  is constitutively expressed, to reduce the risk of an uncontrolled immune responses and autoimmunity [20]. After Food and Drug Administration approval in metastatic renal cancer in 1992 and metastatic melanoma in 1998, it represents the only molecule capable of achieving long-term survival in < 10% of patients with these tumors. It can be used in different doses and modalities of administration, but a high-dose bolus has been shown to be superior to other modalities only in patients attaining complete remission in renal cancer [21]. Furthermore, sporadic activity has been reported in other non-immunogenic tumors [22-24].

Considering the role played by Treg and MDSC on anti-tumor immunosuppression, it is therefore possible to hypothesize that agents capable of negatively modifying these two immunosuppressive cells can improve the antitumor immune activity. In light of the superiority of combination drugs on both immune-suppressive cells [25] we undertook areal-world experience with Cyt alone or in combination with FU, prior to the administration of IL-2, in heavily pre-treated solid tumor patients not susceptible to other potentially effective treatments [2]. From this experience we found a transient decrease of Treg after therapy, especially with intravenous IL-2 administration, a subjective improvement with pain control, objective response on 23% of patients, and a reasonable lengthening in survival. Noteworthy is the temporal disease control with significant symptomatic improvement achieved for the first time with this chemo-immunotherapeutic combination in a patient with very advanced GIST disease. Moreover, the increase and the blood decrease respectively of Treg and NK cells observed in this GIST case could make to hypothesize their opposite representation in the tumor but that should be substantiated from the pathological point of view. However our overall preliminary data require further perspective study in a more selected series with the aim of verifying in a more timely manner the possible temporal variations of lymphocyte subpopulations on the tumoral tissue and on the blood by comparing chemo-immunotherapy with a control group with immunotherapy alone and its correlation with response to treatment and survival.

## Conflicts of Interest

None

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## Abbreviation

GIST: gastro intestinal stromal tumour; CD117: tyrosine-protein kinase Kit; CD34: transmembrane phosphoglycoprotein; S100: S100 protein; KI 67: KI 67 antigen; Cyt: Cyclophosphamide; FU: Fluorouracil; IL-2: Interleukin-2; HPF: high power fields; IM: Imatinib; Tregs: Lymphocyte T regulator cells; CD8: Lymphocyte T CD8; NK: Natural killer; CRP: C-reactive protein; c-KIT: proto-oncogene tyrosine kinase receptor KIT; MCH: major histocompatibility complex; DC: dendritic cell; STS: soft tissue sarcoma; IFN  $\gamma$ : interferon gamma; IKDC: CD11c+ cells IFN-producing killer DC; HLA-DR: MHC class II cell; CD4: Lymphocyte T CD4; CD56: Lymphocyte T CD56; NLR: Neutrophil/Lymphocyte ratio; PD-1: Programmed cell death protein 1; PDL-1: Programmed death-ligand 1; CTLA-4: Cytotoxic T-Lymphocyte Antigen 4; MDSC: myeloid-derived suppressor cells; CD25: Lymphocyte T CD25; IL-2R $\alpha\beta\gamma$ : interleukin-2 receptor  $\alpha\beta\gamma$

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