THE LANCET Oncology

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Zsiros J, Brugieres L, Brock P, et al, for the International Childhood Liver Tumours Strategy Group (SIOPEL). Dose-dense cisplatin-based chemotherapy and surgery for children with high-risk hepatoblastoma (SIOPEL-4): a prospective, single-arm, feasibility study. *Lancet Oncol* 2013; published online July 4. http://dx.doi.org/10.1016/S1470-2045(13)70272-9.

APPENDIX

Dose-dense cisplatin-based chemotherapy and surgery for children with high-risk hepatoblastoma (SIOPEL-4): a prospective, single arm, feasibility study.

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Supplementary methods:

Treatment

Treatment consisted of three cycles of pre-operative chemotherapy (cycles A1, A2 and A3) followed by surgical removal of all remaining tumour lesions if feasible (including liver transplantation (LTX) and/or metastasectomy, if needed) followed by post-operative chemotherapy (cycle C). Patients whose tumour remained unresectable received additional chemotherapy (cycle B) before surgery was attempted. In these cases no post-operative chemotherapy was given . No additional therapy was recommended for microscopic residual disease after liver surgery.

Chemotherapy details:

Cycle A1:
cisplatin 80 mg/m^2/day i.v. infusion in 24 hours; *on day 1*
cisplatin 70 mg/m^2/day i.v. infusion in 24 hours; *on days 8 and 15*
doxorubicin 30 mg/m^2/day i.v. infusion in 24 hours; *on days 8 and 9*

The first dose of Cisplatin (day 1) was 80 mg/m²/day in order to have the same dose as in the standard risk protocol SIOPEL 3-SR so that definitive risk allocation and registration on SIOPEL 3 (for standard risk) or SIOPEL 4 (for high risk) would be possible even after the first Cisplatin course, up to day 7 (Figure1).

Cycle A2:
cisplatin 70 mg/m^2/day i.v. infusion in 24 hours; *on days 1, 8 and 15*
doxorubicin 30 mg/m^2/day i.v. infusion in 24 hours; *on days 8 and 9*

Cycle A3:
cisplatin 70 mg/m^2/day i.v. infusion in 24 hours; *on days 1 and 8*
doxorubicin 30 mg/m^2/day i.v. infusion in 24 hours; *on days 1 and 2*

Cycle A3 was modified (number of Cisplatin doses and timing of Doxorubicin) in order to avoid delay in surgery due to the bone marrow toxicity of this course (Figure 1).

The following recommendations were given regarding the start of cycles (blocks) A2 and A3:
'Before starting the block patients should have recovered from the previous course and must not have signs of active infection. Absolute neutrophil count (ANC) and platelet count should have recovered to above 1x10^9/l and 100x10^9/l, respectively. It is better to delay chemotherapy (with a maximum delay of two weeks) until these criteria are met, rather than decrease the dose. If the block has been started it should be completed regardless of the blood cell count during the course. The only reason for stopping treatment would be grade 4 or life threatening toxicity.'

Cycle B:

doxorubicin 25 mg/m^2/day i.v. infusion in 24 hours; *on days 1, 2, 3, 22, 23 and 24*
carboplatin AUC 10.6 mg/ml.min/day i.v. infusion in 1 hour; *on days 1 and 22*

Block B was given for selected patients as soon as the patient has recovered from the last chemotherapy course according to the following recommendation: 'Patients must not have signs of active infection and absolute neutrophil count (ANC) and platelet count should have recovered to above 1x109/l and 100x109/l respectively. It is better to delay chemotherapy (with a maximum delay of two weeks) until these criteria are met, rather than decrease the dose'. (Figure 2)

Cycle C:
doxorubicin 20 mg/m^2/day i.v. infusion in 24 hours; *on days 1, 2, 22, 23, 43 and 44*
carboplatin AUC 6.6 mg/ml.min/day i.v. infusion in 1 hour; *on days 1, 22 and 43*

Block C was given post-operatively for eligible patients as soon as the patient has recovered from surgery according to the following recommendation: 'Patients must not have signs of active infection. An absolute neutrophil count (ANC) greater than 1x109/l and a platelet count greater than 100x109/l are necessary before starting chemotherapy. It is better to delay chemotherapy (with a maximum delay of two weeks) until these criteria are met, rather than decrease the dose'. (Figure 3)

Dose modifications
In the protocol the following recommendations were given for dosis modifications:

Dose modification by age:
- For infants and children with body weight between 5 and 10 Kg: Cisplatin and Doxorubicin doses should be calculated per Kg, assuming that 1m^2=30Kg.
- For infants with body weight less than 5 Kg: Cisplatin and Doxorubicin doses should be calculated per Kg, assuming that 1m^2=30Kg, and a further 1/3 dose reduction should be applied.

Drug	children > 10 Kg	children 5-10 Kg	children < 5 Kg
Cisplatin:	70 mg/m^2/day	2.3 mg/Kg/day	1.5 mg/Kg/day
Cisplatin:	80 mg/m^2 day 1	2.7 mg/Kg day 1	1.8 mg/Kg day 1
Doxorubicin:	20 mg/m^2/day	0.67 mg/Kg/day	0.44 mg/Kg/day
Doxorubicin:	25 mg/m^2/day	0.83 mg/Kg/day	0.56 mg/Kg/day
Doxorubicin:	30 mg/m^2/day	1.0 mg/Kg/day	0.67 mg/Kg/day

Dose modification by toxicity:
- Bone marrow toxicity:

An absolute neutrophil count (ANC) greater than 1x10^9/l and a platelet count greater than 100x10^9/l are necessary before starting each course. It is better to delay chemotherapy until these criteria are met (for a maximum delay of two weeks), rather than decrease the dose. If a delay of one or more weeks is necessary, decrease dosage by 25% for the next course. If a course of chemotherapy results in severe neutropenia (ANC < 0.5x10^9/l) associated with fever and sepsis or severe infection and/or severe thrombocytopenia associated with bleeding, decrease dosage by 25% for the next course. If the subsequent course is not complicated by these events resume the full dosage of the drugs.
Growth factor support: The use of G-CSF between the cycles of chemotherapy is recommended where available. G-CSF should not be given during chemotherapy or 24 hours prior to or following chemotherapy. The most useful time for the administration of G-CSF will be between cycles. The recommended dose is 5µg/kg/day given subcutaneously or IV.

- Ototoxicity:

If grade 3 ototoxicity is documented please contact the chemotherapy panel co-ordinators.

- Renal toxicity:

In cases of severe reduction in 51CrEDTA GFR (<60 ml/min/1.73 m2), discontinue Cisplatin and contact the chemotherapy panel co-ordinators. In infants refer to Appendix 10 for the curve of expected GFR according to age. If GFR falls 2 SD below the expected GFR contact the chemotherapy panel co-ordinators. Hypomagnesemia is not a reason to stop Cisplatin. However in infants electrolyte disturbances are to be expected and can lead to fitting particularly if there are multiple abnormalities. The infusion guidelines are standard, however, in some children the electrolyte substitution may need to be increased. Please monitor electrolytes carefully.

- Hepatotoxicity:

No standardised criteria exist for the adjustment of Doxorubicin dosage in the presence of hepatic dysfunction. It seems reasonable to recommend a 50% reduction of Doxorubicin dosage if total serum bilirubin concentration > 3 mg/100 ml or/and if serum liver enzymes (AST, ALT) are >5 times the normal value. Cisplatin dose need not be modified. NB. Since Doxorubicin hepatotoxicity is actually very rare, elevated AST, ALT should lead to a vigorous search for other causes of liver dysfunction, eg. viral hepatitis.

- Cardiotoxicity

Significant deterioration in function is indicated by a shortening fraction <29%. In this event, temporarily withdraw Doxorubicin. If subsequent testing shows an improvement in shortening fraction, consider reintroducing Doxorubicin. A fall in shortening fraction by an absolute value of > 10 percentile units but with an actual SF value > 29% (eg SF 42% $\rightarrow$ SF 31%) may also represent a significant deterioration in function. In this event contact the chemotherapy panel co-ordinators.'

Surgical guidelines

The following guidelines were given in the protocol regarding surgical assessment and treatment:

Definitive tumour resection

- Timing of definitive surgery:

Definitive surgery should preferably take place after three blocks of pre-operative chemotherapy (blocks A1-A3). At this point every attempt should be made to remove the tumour completely (without microscopic residual disease) through partial or total hepatectomy. However, if complete resection (including liver transplant) is deemed not feasible at this point (e.g. because of persistence of abdominal extra-hepatic disease or unresectable pulmonary metastases) patients should first proceed to additional chemotherapy. After block B, resectability must be reassessed and definitive surgery, if feasible, should then take place.Tumour resection should be performed as soon as the patient has recovered from the last chemotherapy course.

- Assessment of resectability:

An accurate assessment requires optimal imaging studies: spiral CT followed by contrast administration (including angio-CT reconstruction of hepatic vessels, when necessary) and/or magnetic resonance imaging with Gadolinium administration. Ultrasonographic Doppler examination is of particular value allowing real-time investigation of the tumour and its relation to hepatic vessels including assessment of their patency or eventual invasion. However, distinction between real invasion beyond the anatomic border of a given sector and displacement can be very difficult indeed. Tumour resectability also depends on surgical expertise; for instance, some tumours involving both liver lobes can still be radically resected by trisegmentectomy, when one lateral sector is disease-free. Even tumour encasement or ingrowth into the retrohepatic vena cava does not preclude a radical excision since it can be resected "en-bloc" and replaced by either a "Goretex" prosthetic graft, or a venous allograft. In selected and rare cases declaration of the tumour's inoperability may require a diagnostic laparotomy, which should be performed at the transplant centre, if at all. In the majority of cases it should be discouraged because it is usually more difficult to assess resectability intraoperatively than before the operation by an experienced radiologist with the surgeon sitting by.

- Issue of "difficult" liver resections:

Generally, very difficult liver resections carrying a high probability of leaving tumour residue should be avoided, whenever possible. This rule applies mainly to the tumours located in close proximity to the major hepatic vessels, which in order to be preserved would have to be peeled off the tumour. In such children first line liver transplantation should be considered. In cases, when difficult hepatic resection is planned or when liver transplantation has to be delayed, hepatic artery chemoembolization (HACE) may be considered. In most cases this can achieve a decrease in the tumour size and significant necrosis, even when the tumour has not responded to former systemic chemotherapy. It also results in the formation of a thick fibrous capsule around the mass, as well as its calcification, which further facilitates the resection, particularly in the vicinity of the vessels. Completeness of the tumour resection should be assured during hepatectomy by all possible means. If there are any doubts frozen sections of the resection margin should be obtained. Also the resection margin on the patient's side should be sampled and immediately inspected by a pathologist, when possible and applicable. When microscopic residue is found, the surgeon should explore the possibility of immediate reresection of the margin taking an "extra slice" of the liver, when technically applicable and reasonable. However, the final judgement of the completeness of resection is dependent on the final pathology report. For

more detailed advice regarding difficult liver resections and specific surgical techniques, including HACE, please contact the surgical co-ordinator or members of the surgical panel.

Liver transplantation

- Possible indications for liver transplantation:

In the following cases total hepatectomy with liver transplantation must be considered very seriously. Accurate assessment of resectability, facilitated by optimal imaging studies, must take place both at the diagnosis and after neo-adjuvant chemotherapy. Because of the increased likelihood of liver transplant, advice of a paediatric liver transplant surgeon should be sought as early as possible, i.e. directly at the beginning of treatment. Close contact between the surgeons, radiologist and oncologist should be maintained throughout the pre-operative chemotherapy in order not to miss optimal timing for transplantation. The definitive decision on the surgical technique to be used and the necessity of liver transplantation must be taken in a comprehensive discussion by a transplant surgeon, liver surgeon, radiologist and paediatric oncologist.

  - Large, solitary PRETEXT IV tumour (involving all four sections of the liver at presentation, as confirmed by state-of-the art imaging): unless tumour "down-staging" is clearly demonstrated after pre-operative chemotherapy (as could be the case when the anatomic border of a normal liver sector is compressed without true malignant invasion), primary liver transplantation seems to be the best option.
  - Multifocal PRETEXT IV tumour: even in the case of good response to chemotherapy accompanied by down-staging of the tumour (disappearance of tumour lesions from at least one hepatic sector), it is recommended to perform total hepatectomy followed by liver transplantation in order to guarantee resection of all (remaining) lesions, including the microscopic ones that could not be visible (any more) on imaging studies.
  - Unifocal centrally located tumours involving main hilar structures or main hepatic veins, as can be the case for some PRETEXT II or III V+/P+ tumours: it is highly probable that these tumours will not become resectable with partial hepatectomy even after good response to chemotherapy because of the localisation of the tumour.

N.B.:U Initial portal (P) or hepatic veins/vena cava (V) involvement is not a contraindication for eventual liver transplantation. Even if the vascular involvement persists after chemotherapy, it should not be considered as an absolute contraindication for transplant. However in these patients retro-hepatic VC and as much portal vein as possible should be removed with en bloc liver. Such cases should be individually discussed with the transplant surgeon and/or transplant protocol coordinator. In any case when a surgeon has doubts as to the feasibility of surgical resection it is strongly recommended that the advice of a paediatric liver transplant surgeon and/or of the transplant/surgical co-ordinator of the trial is sought.

- Contraindications:
  - Persistence of viable extra-hepatic deposits, not amenable to surgical excision, is an absolute contraindication to LTX.
  - Poor tumour response (stable disease or progression) to preoperative chemotherapy is also a contraindication to liver transplantation.
- Timing of LTX:

The timing of definitive surgery, including liver transplantation, is crucial for achieving adequate local tumour control. LTX should not be delayed in excess of four weeks after the last course of chemotherapy. Entry on the cadaveric waiting list is a good option if the access to a donor graft can be expected within this time. Otherwise intra-familial live donor liver transplant must be considered.

- Technical considerations:

For the sake of complete tumour excision, it is advocated that total hepatectomy for primary malignancies in children should include removal of the retrohepatic inferior vena cava. This approach does not preclude a cadaveric transplant provided with retrohepatic vena cava. In a live related liver transplant not provided with vena cava, reconstruction can be achieved either with a preserved allogeneic iliac vein procured from a cadaveric donor or, preferentially, with the internal jugular vein procured from the parental donor. The first choice, however, should be IVC replacement with the left internal jugular vein of the patient (alternatively with the external iliac vein). In this case, the patient should be treated with low molecular weight heparin and wear elastic socks. Autologous vein is superior to venous allograft, which will often get sclerosed and obstructed, while a Goretex graft will become too small with growth of the patient and will thrombose with time.

- Liver transplant in children with initial lung metastasis:

Based on currently available data it appears that liver transplant is a viable option for children presenting with lung metastases. However, all metastatic lesions must be either eradicated by chemotherapy alone or (post-chemotherapy remnants) must be removed by surgical resection, beforehand. Tumour-free condition of the lungs must be confirmed with advanced imaging studies.

- Second line liver transplantaion:

Since the results of primary LTX are by far superior, in terms of patient survival, to those of rescue LTX, heroic attempts at partial hepatectomy with risk of incomplete resection, should be avoided.

Surgery in patients with initial lung metastases:

In general, the presence of lung metastases at diagnosis is not a contraindication for partial liver resection or liver transplantation. Some lung metastases respond very well to chemotherapy and can disappear completely or become resectable by the end of the preoperative chemotherapy. Removal of pulmonary residual disease with subsequent resection of the primary tumour is then considered a valid and curative option. We must, however, emphasize that the role of liver transplant in this setting is still to be established. Accordingly,

- if metastases have disappeared by the end of pre-operative chemotherapy (blocks A1-A3), all attempts should be made to resect the primary tumour completely either by partial hepatectomy or by LTX. The disappearance of the metastases must, however, be confirmed by appropriate imaging studies (CT scan).
- if metastases are not (yet) resectable after blocks A1-A3, the patient should receive additional pre-operative chemotherapy (block B). Resectability should than be reassessed and an attempt at complete tumour removal should be made if deemed feasible.
- if metastases become resectable by the end of pre-operative chemotherapy, 'aggressive multiple surgery' is recommended to resect both pulmonary metastases and the primary tumour. The complete removal of pulmonary metastases must be confirmed with appropriate imaging studies (CT scan) prior to resection of the primary tumour. We recommend, however, that in the case of continuous tumour response to the first three blocks of pre-operative chemotherapy (A1-A3), the use of block B can be considered before surgery in the hope that further chemotherapy may induce the complete disappearance of lung lesions. However, in this case the tumour volume of the primary and lung lesions and the serum AFP value should be carefully monitored to document a continuous tumour response to chemotherapy and avoid progression. Definitive surgery should than be performed after block B.

In patients who undergo both pulmonary and abdominal surgery, administration of chemotherapy can be considered between the two operations to guarantee continuous chemotherapeutic control of tumour growth until all tumour lesions have been removed. For this purpose (a part of) the post-operative chemotherapy block (block C) is recommended (Carboplatin+Doxorubicin). The number of courses (1-3) should be individually chosen, depending on the tumour situation, recovery time and waiting time for surgery. The remaining courses (0-2) should be given post-operatively.

Supplementary results:

Chemotherapy:

The interval between the administration of the first cisplatin in A1 (day 1 of protocol) and the start of cycle A3 (day 57 of the protocol) was: 53 -120 days (range), median: 64 days, mean: 67 days.
Dosis reduction was done in one of the 62 A1 cycles (in a severely ill child with multi organ problems), in four of the 61 A2 and in seven of the 59 A3 cycles (all due to previous haematological toxicity). Of the 37 C cycles in 8 the dosis was at least partly reduced due to haematological toxicity. All the 13 B cycles were administered without dosis reduction.

Surgery:

Of the seven patients in whom metastectomy was performed in five this operation was carried out before the liver surgery with a interval of 2, 4, 4, 6 and 20 weeks, respectively, between the two operations. In two patients metastectomy and liver surgery were done in one session.

Toxicity:

List of serious adverse events:
grade: 3 category: AUDITORY/EAR
grade: 3 category: AUDITORY/EAR
grade: 1 category: METABOLIC/LABORATORY:subcat:Glomerular filtr. rate
grade: 3 category: AUDITORY/EAR
grade: 3 category: AUDITORY/EAR
grade: 4 category: INFECTION, subcategory:Febrile neutropenia
grade: 4 category: METABOLIC/LABORATORY,subcategory: hypophosphatemia
grade: 4 category: METABOLIC/LABORATORY, hypokalemia
grade: 3 category: PULMONARY/UPPER RESPIRATORY,pulmonary infiltrates
grade: 2 category: LYMPHATICS,subcategory: Lymphocele
grade: 2 category: ALLERGY/IMMUNOLOGY, subcategory: Allergic reaction
grade: 1 category: DERMATOLOGY/SKIN,subcategory: extravasation
grade: 3 category: CONSTITUTIONAL SYMPTOMS, subcategory: Weight loss
grade: 3 category: INFECTION
grade: 3 category: AUDITORY/EAR
grade: 3 category: INFECTION
grade: 5 category: HEMORRHAGE/BLEEDING in tumour
grade: 5 category: INFECTION

Figure 1.

PRE-OPERATIVE CHEMOTHERAPY

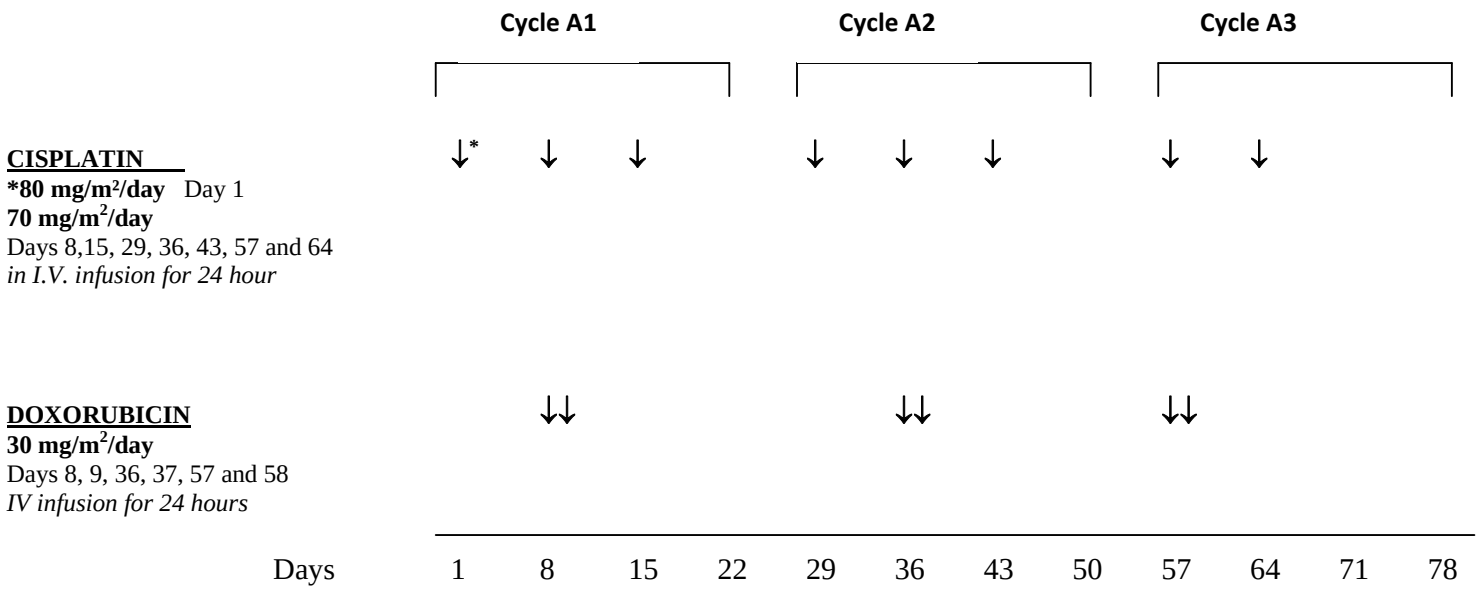


Figure 2.

ADDITIONAL PRE-OPERATIVE CHEMOTHERAPY

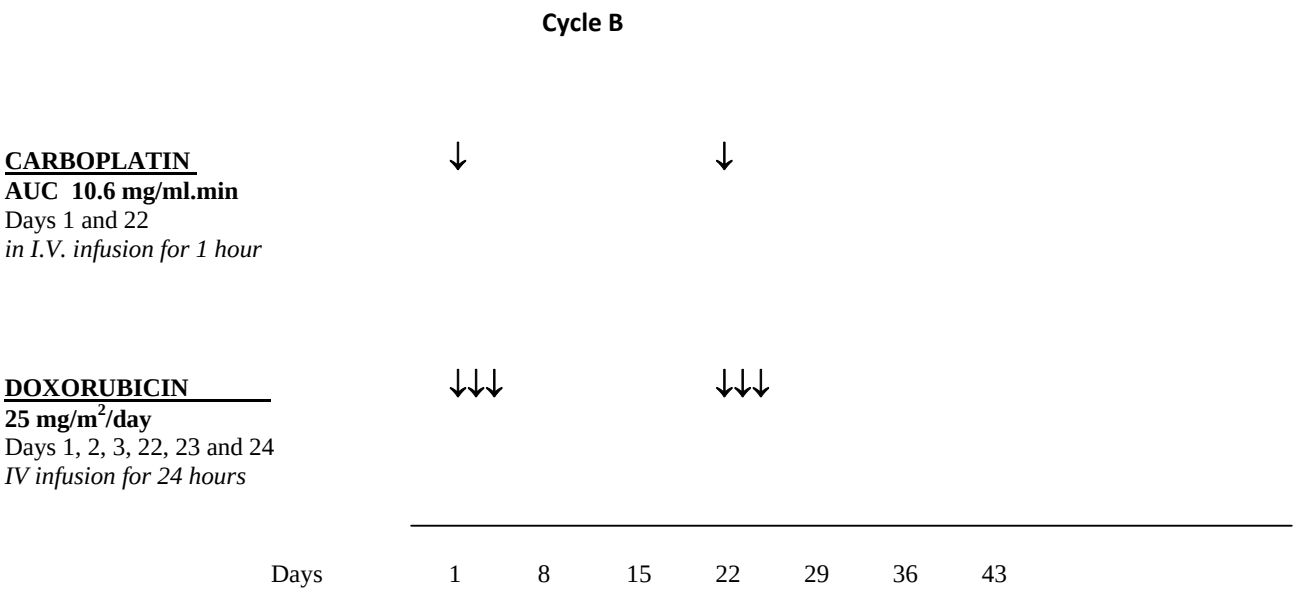
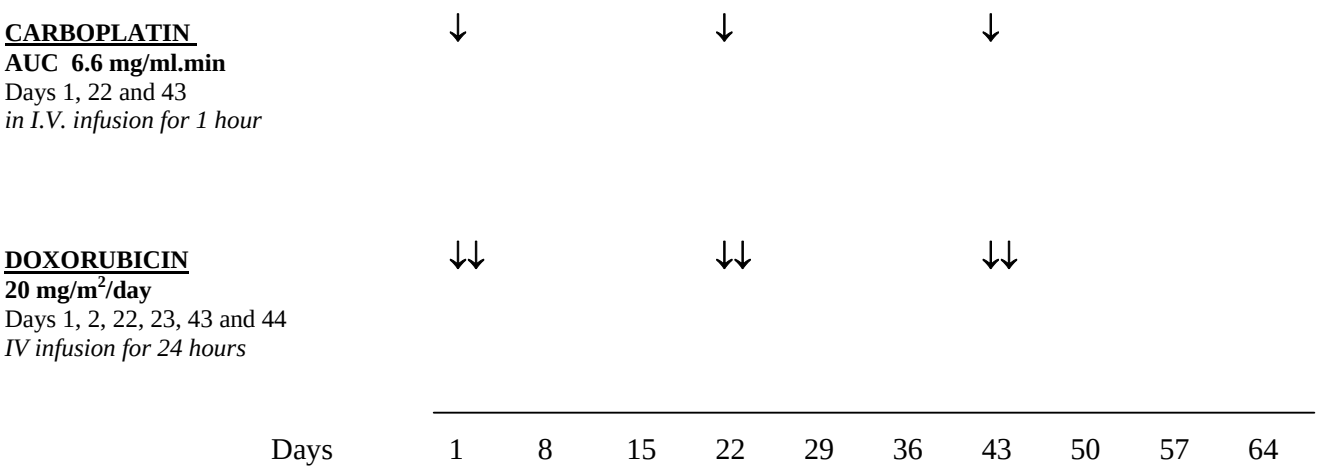


Figure 3.

POST-OPERATIVE CHEMOTHERAPY

Cycle C



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