UNIVERSIDAD DE SEVILLA

FACULDAD DE MEDICINA

Departamento de Cirugía

Tesis doctoral

Análisis obsevacional para evaluar la eficacia y seguridad de los mTORi en la esfera oncológica en el paciente trasplantado hepático

Presentada por

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SEVILLA, 2019

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CERTIFICA:

Que la Tesis Doctoral que lleva por título: **"Análisis obsevacional para evaluar la eficacia y seguridad de los mTORi en la esfera oncológica en el paciente trasplantado hepático"**, presentada por D. XI DANG para optar al grado de Doctor, ha sido realizada en el Departamento de Cirugía de la Facultad de Medicina de la Universidad de Sevilla.

Revisado el texto, doy mi conformidad para su presentación y defensa para optar al grado de Doctor por la Universidad de Sevilla.

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3 de Junio de 2018

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Agradecimiento

A Dr. Luis Cristóbal Capitán Morales, tutor de esta tesis, por haberme brindado la oportunidad de este estudio, por su apoyo incondicional y motivación.

A Dr. José María Álamo Martínez, director de esta tesis, por su gran paciencia y enorme capacidad de esfuerzo, por sus ganas de aprender y enseñar al mismo tiempo.

A Dr. Miguel Ángel Gómez Bravo, director de esta tesis, por su apoyo y su implicación en mi formación médica e investigadora.

A Dres. Luis Miguel Marín, Ganzalo Suarez, Carmen Bernal, Carmen cepeda, integrantes de la Unidad de Cirugía Hepatobiliopancreática y Trasplante. Por sus introducción y enseñanza en la cirugia hepatobiliopancreática, por su confianza y apoyo.

A mi familia.

Abstract

Background: the liver transplant patient has an extraordinary connection with the oncological sphere. On the one hand, an increasingly frequent investigation of liver transplantation is the presence of a hepatocellular carcinoma, and, on the other hand, the appearance of "de novo neoplasms" favored by the state of immunosuppression to which these patients are subjected. This close relationship with cancer is a frequent cause of death. The immunosuppressant treatment based on calcineurin inhibitor (CNI, tacrolimus, cyclosporine), controls the appearance of rejection but favor the appearance and proliferation of neoplastic cells by relaxation of the immune tumor surveillance. The appearance of mTOR inhibitors (mTORi, sirolimus, everolimus) within the immunosuppressive arsenal as non-nephrotoxic agents may represent an advance in the tumor control of these patients, since they are basically used in the area of oncology as antiproliferative agents.

Objectives: 1): To analyze if the use of mTORi in the liver transplant patient by HCC reduces the incidence of recurrence and increases survival. 2): To analyze whether the use of mTORi in the liver transplant patient with de novo neoplasia reduces the incidence of recurrence and increases survival.

Methodology: retrospective observational analysis of cases (patients with de novo neoplasms or those undergoing hepatic transplantation due to immunosuppressed hepatocarcinoma with mTOR inhibitors) and controls (immunosuppressed with calcineurin inhibitors), analyzing the rate of recurrence and cumulative survival.

Patients: 392 patients were selected, analyzing cases and controls in two groups: patients transplanted for hepatocellular carcinoma (HCC) and patients in whom some de novo neoplasia (NN) appeared in their evolution.

Results: In the HCC group, alcohol and tobacco consumption increase the incidence of HCC. (Alcohol: $p = 0.035$, Tobacco: $p = 0.67$). Vascular and capsular invasion and the Edmondson grade reduce survival (Vascular invasion: $p = 0.00$, appearance of the capsule: $p = 0.00$, Edmondson: $p = 0.09$).

The use of CNI increases the incidence of recurrent HCC ($p = 0.025$). In comparison with the CNI, the mTORi increase the survival of these patients ($p = 0.05$). As for the NN group, alcohol consumption and tobacco consumption reduce survival after tumor diagnosis (alcohol: $p = 0.02$, tobacco: $p = 0.001$). The resection of NN, in comparison with chemotherapy and radiotherapy, increases survival ($p = 0.00$). The tumor stage and TNM is directly related to patient survival ($p = 0.000$). The use of mTORi in the immunosuppressive regimen increases the survival of liver transplant patients with de novo neoplasia compared to the use of CNI ($p = 0.067$).

Resumen

Antecedentes: el paciente trasplantado hepático presenta una extraordinaria conexión con la esfera oncológica. Por un lado, una indacación cada vez más frecuente de trasplante hepático es la presencia de un hepatocarcinoma, y, por otro lado, la aparición de “neoplasias de novo” favorecidas por el estado de inmunosupresión al que están sometidos estos pacientes. Esta estrecha relación con el cáncer supone una causa frecuente de muerte. El tratamiento inmunosupresor basado en anticalcineurínico (CNI; tacrolimus, ciclosporina), controlan la aparición de rechazo pero favorecen la aparición y proliferación de las células neoplásicas por relajación de la vigilancia tumoral inmunitaria. La aparición de inhibidores de mTOR (mTORi; sirolimus, everolimus) dentro del arsenal inmunosupresor como agentes no nefrotóxicos pueden suponer un avance en el control tumoral de estos pacientes, ya que se utilizan básicamente en el área de la oncología como agentes antiproliferativos.

Objetivos: 1): Analizar si el uso de mTORi en el paciente trasplantado hepático por CHC, reduce la incidencia de recidiva y aumenta la supervivencia. 2): Analizar si el uso de mTORi en el paciente trasplantado hepático con neoplasia de novo, reduce la incidencia de recidiva y aumenta la supervivencia.

Metodología: análisis observacional retrospectivo de casos (pacientes con neoplasias de novo o aquellos sometidos a trasplante hepático por hepatocarcinoma inmunodeprimidos con inhibidores de mTOR) y controles (inmunodeprimidos con inhibidores de la calcineurina), analizando la tasa de recidiva y supervivencia acumulada.

Pacientes: Se seleccionaron 392 pacientes, analizando casos y controles en dos grupos: pacientes trasplantados por hepatocarcinoma (CHC) y pacientes en los que apareció en su evolución alguna neoplasia de novo (NN).

Resultado: En el grupo de HCC, el consumo de alcohol y el tabaco aumentan la incidencia de HCC. (Alcohol: $p = 0.035$, Tabaco: $p = 0.67$). La invasión vascular y de la cápsula y el grado de Edmondson reducen la

supervivencia (Invasión vascular: *p*=0.00, apariencia de la cápsula: *p*=0.00, Edmondson: *p*=0.09). El uso de CNI aumenta la incidencia de HCC recurrente (*p*=0,025). En comparación con la CNI, los mTORi aumentan la supervivencia de estos pacientes (*p*=0.05). En cuanto al grupo NN, el consumo de alcohol y el consumo de tabaco reducen la supervivencia tras el diagnóstico del tumor (alcohol: p = 0.02, tabaco: p = 0.001). La resección de la NN, en comparación con la quimioterapia y la radioterapia aumentan la supervivencia (p = 0,00). El estadío tumoral y TNM se relaciona directamente con la supervivencia del paciente (p = 0,000). El uso de mTORi en el esquema inmunosupresor aumenta la supervivencia de los pacientes trasplantados hepáticos con neoplasia de novo en comparación con el uso de CNI (p = 0.067).

Conclusiones: El tabaco y el alcohol reducen la supervivencia de los pacientes después del TOH en el HCC y la neoplasia de novo. La invasión vascular y capsular y grados avanzados de Edmondson reducen la supervivencia de los pacientes trasplantados debido a HCC. La inmunosupresión con mTORi, especialmente el everolimus, aumenta la supervivencia de los pacientes después del TOH en el HCC en comparación con la CNI. Para el cáncer sólido, la resección aumenta la supervivencia de los pacientes con TOH con neoplasia de novo en comparación con quimioterapia / radioterapia. TNM y el estadio tumoral son buenos predictores de supervivencia. La inmunosupresión mTORi, especialmente everolimus, aumenta la supervivencia de los pacientes después del TOH en la neoplasia de novo en comparación con la CNI, sobre todo en el cáncer ORL, cáncer de pulmón, síndrome linfoproliferativo, cáncer digestivo, cáncer de mama y cáncer urinario. Este beneficio de mTORi no se puede aplicar al cáncer de piel ni al cáncer hepatobiliopancreático. La monoterapia con MMF también aumenta la supervivencia en ORL y cáncer de mama

Abbreviations

AZA …….…… Azathioprine

CNI …….. Calcineurin inhibitor

DM ……..…… Diabetes Mellitus

EVR …… Everolimus

FDA ……..... Food drug administration

HCC …….. Hepatocellular carcinoma

mTOR ……..… The target of mammalian rapamycin

mTORi ……..…… The target of mammalian rapamycin inhibitor

MELD ……... Model for End-stage Liver Disease

MMF ……..…….. Mycophenolate mofetil

NN ……. *De novo* neoplasia

SRL ……... Sirolimus

TAC …… Tacrolimus

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1. INTRODUCTION

1. INTRODUCTION

1.1. History of liver transplantation

Liver transplantation (LT) is considered to be the treatment used in patients with acute or chronic liver disease when other alternative therapy has been exhausted and it is the best treatment for end-stage chronic liver disease and is associated with a one-year survival rate that ranges from 78% to 85%[1].

The research possibility of LT began in the 1960s. In 1955, Dr. Welch was the first to describe liver transplantation as a treatment and proposed ectopic liver transplantation in the abdominal cavity. However, modern liver transplantation is orthotopic liver transplant (OLT), which consists of excision of the diseased liver followed by placement in the same anatomical location of a healthy liver from an organ donor, constituted by grafting the entire or a part of a liver[2]. A few later, in 1963, Starzl et al attempted the first human LT in the world, the patient was a 3-year-old boy with biliary atresia, who died during surgery due to coagulation disorder and uncontrolled bleeding[3],[4]. Especially, using the same immunosuppressive regimen, at the University of Cambridge, United Kingdom, 138 cases of OLT were performed between 1968 and 1984, and the results were so disappointing[5],[6],[7].

In 1990, Starzl et al.[8]. reported the first use of the new tacrolimus as imunosupressant agent in patients submitted to liver transplantation who suffered a rejection even using conventional immunosuppressive treatment. Tacrolimus, which is similar to CsA but with greater potency. The success of cyclosporine conversion by tracrolimus in these patients and by showing it safety and effectiveness of rejection control, lots of new clinical studies were conducted with using tacrolimus as the main immunosuppressor in liver transplantation[8910]. Until then, the CNIs (Cyclosporine A and Tacmolimus) had been the basis immunosuppressor in OLT. At the end of the 1990s,

a new purine synthesis inhibitor was introduced, mycophenolate mofetil (MMF), which was presented as a more selective alternative and with greater immunosuppressive capacity than azathioprine (AZA).

In fact, CNIs presents nephrotoxic effects, which are an important source of mortality in OLT. Almost 20% of OLT recipients have chronic renal failure after 5 years of the transplantation[11]. Everolimus (EVE) and Sirolimus (SRL), two new immunosuppressant of the mammalian Target Of Rapamycin Inhibitor (mTORi), could potentially satisfy this adverse effect. The chief advantage sirolimus has over calcineurin inhibitors is its low toxicity toward kidneys. Transplant patients maintained on calcineurin inhibitors long-term tend to develop impaired kidney function or even chronic renal failure; this can be avoided by using sirolimus instead. It is particularly advantageous in patients with kidney transplants for hemolytic-uremic syndrome, the SRL was approved by the *Food Drug Administration* (FDA), USA, for kidney transplantation, because it has ability to protect kidney function[1213]. And in the year 2010, the EVE was approved by the FDA being used together with TAC, and reduced dose of TAC and steroid in the OLT recipients to decrease the nephrotoxicity of the CNIs. The SRL has been used in several OLT centers in the world for prophylaxis and to decrease the nephrotoxicity of CNIs.

In Europe, the OLT was widely applied in the 1980s, where the countries that made their first OLT succeeded. (Figure 1.1) According to the information of the European Liver Transplant Registry (ELTR), until the present time, 174 transplant centers from 32 European countries are participating to ELTR. By comparison with the official data Transplant Observatory, More than 98% of European liver transplantation's data are continuously updated, analyzed and published by ELTR.

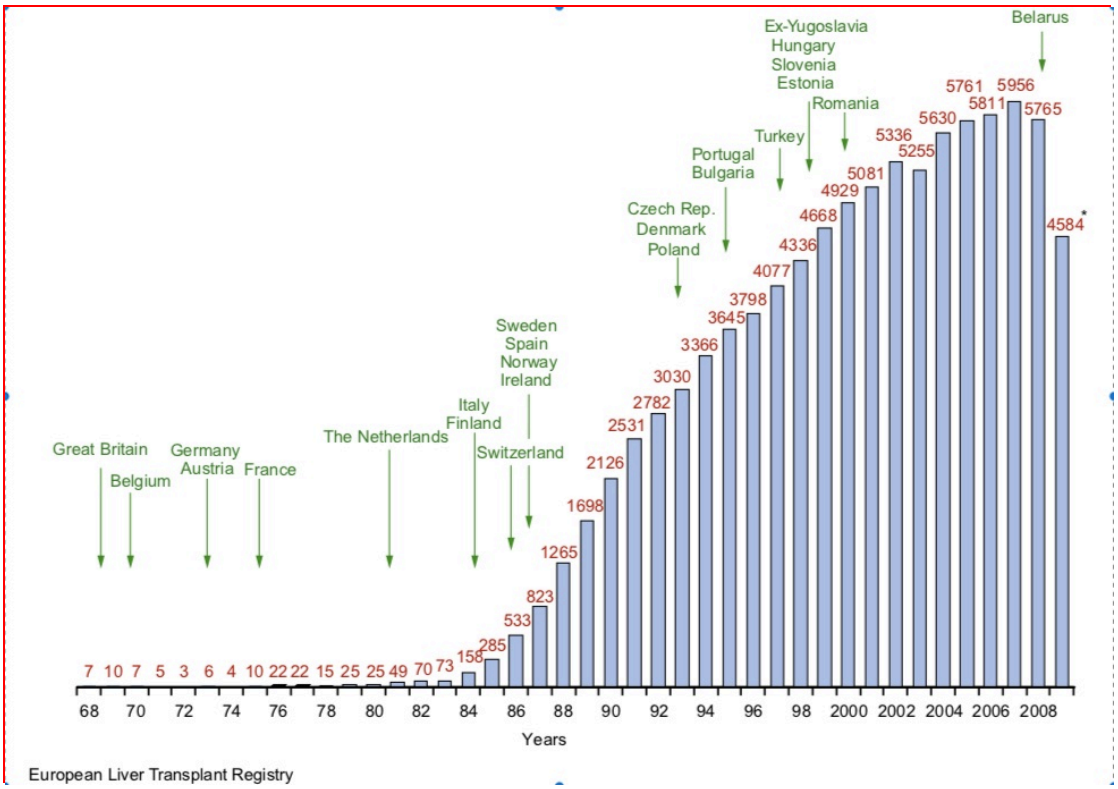


Fig.1.1: Evolution of 93,634 LTs performed in Europe since May 1968.

Arrows indicate the year the first LT was performed in indicated countries. This decrease is owed to the fact that some centers did not yet send their updating further to the recent changes of the questionnaire.

In Spain, the first OLT was successfully carried out in 1984 in Barcelona. The annual number of OLT performed has progressively increased, reaching 1,000 cases per year in the last 10 years. The high activity of OLT is associated with a high rate of liver donation. Spain has an index of 14 donors per million inhabitants, which is the highest, not only in Europe but also in the world.

For now, almost 50 years after the first OLT, the survival rates of 1 and 5 years are 85% and 73%, respectively[14]. This success is related to advances in surgical technique, progress in immunosuppression, progress in graft conservation and anesthetic technique, and advances in perioperative care.

1.2. Indication and contraindication of liver transplant

1.2.1. Indication of liver transplant

The principal objectives of OLT are to prolong survival and improve the quality of life of the patient, while also optimizing the use of available organs[15]. In general, OLT is recommended in those patients in whom a possibility of survival without OLT less than or equal to 90% per year is expected, regardless of the etiology of the disease that motivates the OLT.

Recipient candidates must meet three conditions:

- Have incurable and deadly disease in the short term.
- No contraindication
- Be able to understand and accept what the transplant represents as well as the servitude that it entails.

It is understood as an incurable and deadly disease in the short-term that is progressive and irreversible, with little or no response to treatment, with a poor quality of life and that presents a low percentage of survival per year.

List of liver transplant treatment diseases.

1. Chronic liver diseases

- Chokes

  Primary biliary cirrhosis

  Secondary Biliary Cirrhosis

  Primary Sclerosing Cholangitis

  Biliary Atresia

Caroli's disease

Family Cholestatic Syndromes

- Parenchymatous

  Virus-related liver Cirrhosis (B, C, D, ...)

  Alcoholic Liver Cirrhosis

  Drug-induced Liver Cirrhosis

  Autoimmune Liver Cirrhosis

  Cryptogenic Cirrhosis

- Vascular disease

  Venous-Occlusive Disease

  Budd-Chiari Syndrome

  Congenital Hepatic Fibrosis

2. Liver neoplastic diseases

   Hepatocellular carcinoma

   Metastasis of Neuroendocrine Tumors

   Cholangiocarcinoma

   Other Liver Neoplasms

3. Acute or subacute liver failure

   Acute severe liver failure secondary to virus (A, B, C, D, ...)

   Acute severe hepatic insufficiency due to drugs

   Reye's syndrome

   Cryptogenetics

4. Metabolic and genetic diseases

Organic Aciduria

Familial hereditary amyloidosis

Alpha-1 antitrypsin deficiency

Enzymatic deficiencies in the Urea cycles

Deficiencies of coagulation factors

Wilson's disease

Galactosemia

Glycogenosis Storage Type I and IV

Hemochromatosis

Hemophilia A and B

Homozygous type II hyperlipoproteinemia

Protoporphyria

Tyrosinemia

Crigler-Najjar syndrome type I

Sanfilippo syndrome

Liver neoplastic diseases

Hepatocellular carcinoma(HCC): is a primary malignancy of the liver and occurs predominantly in patients with underlying chronic liver disease and cirrhosis. most OLT centers agree to use the Milan Criteria, which consist of patients with a single tumor less than or equal to 5 cm, or having a maximum of 3 nodules, all of which are less than or equal to 3 cm in diameter; regardless of the Child-Pugh stage, formally excluding patients with extrahepatic extension, multicentric tumors and / or macroscopic vascular invasion.

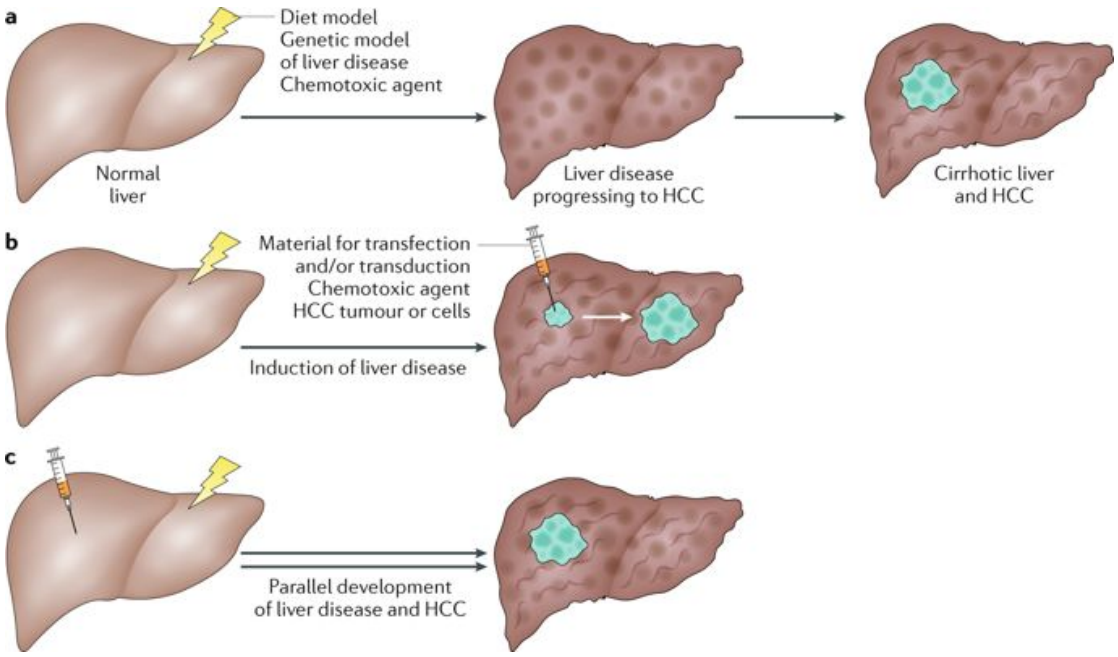


Fig 1.2 The processing of Hepatocellular carcinoma

Cholangiocarcinoma: due to the high rate of recurrence after OLT, traditionally it is not considered an indication of OLT, however, some researchers have suggested that a standard protocol involving external beam and endoluminal radiation and systemic chemotherapy, with lymphadenectomy, may be suitable candidates for OLT[16].

Fibrolamellar carcinoma and Neuroendocrine tumors metastatic to the liver: (gastrinoma, insulinomas, glucagonomas, somatostatinoma and carcinoid tumors): they usually settle on the healthy liver. If these tumors are very extensive and the functional capacity of the liver is damaged after resection, it will be indicated OLT. The recipients should be well selected: young people with massive liver metastases and resected primary tumor, in which the symptoms produced by the metastases are not controlled with medical treatment; OLT in these cases is more a palliative treatment than a cure, due to the high rate of recurrences.

1.2.2. Liver transplant procedure

In patients with chronic liver disease is evaluated mainly with models that predict survival in the absence of transplantation.

There are two prognostic models to evaluate the survival of patients with chronic end-stage liver disease: the ***Child-Pugh index*** and the ***MELD score*** (Model for End-stage Liver Disease).

The Child-Pugh index

This most commonly used prognostic index consists of 5 variables, of which 3 reflect liver function (albumin, bilirubin and prothrombin time) and 2 refer to complications of the disease (ascites and encephalopathy). (Table 1.1-2)

Measure	1 point	2 points	3 points
Total bilirubin, μmol/L (mg/dL)	<34 (<2)	34–50 (2–3)	>50 (>3)
Serum albumin, g/dL	>3.5	2.8–3.5	<2.8
Prothrombin time, prolongation (s) **OR** INR	<4.0 <1.7	4.0–6.0 1.7–2.3	> 6.0 >2.3
Ascites	None	Mild (or suppressed with medication)	Moderate to severe (or refractory)
Hepatic encephalopathy	None	Grade I–II	Grade III–IV

Table 1.1: The score employs five clinical measures of liver disease. Each measure is scored 1-3, with 3 indicating most severe derangement

Points	Class	One-year survival	Two-year survival
5–6	A	100%	85%
7–9	B	80%	60%
10–15	C	45%	35%

Table 1.2: Chronic liver disease is classified into Child-Pugh class A to C

The total score of 5-6 is considered grade A (well compensated disease); 7-9 is grade B (significant functional commitment); and 10-15 is grade C (decompensated disease). These grades correlate with a patient's survival at 1 or 2 years.

The Child Pugh index is very easy to determine, but there are drawbacks. Such are that it has not been obtained in multivariate analysis; the assessment of the degree of ascites and encephalopathy is subjective; It does not discriminate when the disease is very advanced since it has fixed limits for each variable. Despite its drawbacks, it is still used to assess the indication for liver transplantation, which is considered indicated in Child B and C patients.

MELD Score

MELD (Model for End-stage Liver Disease) was originally developed at the Mayo Clinic by Dr. Patrick Kamath, and at that point it was called the "Mayo End-stage Liver Disease" score. The MELD Score is based on statistical formulas for predicting the risk of death in a short period of time (3 months).

The MELD is calculated from laboratory parameters:

- Bilirubin
- INR (international normalized ratio) of prothrombin.
- Creatinine

The MELD value is calculated with a formula based on Neperian logarithms of the mentioned parameters:

MELD = 3.78 × ln [serum bilirubin (mg / dL)] + 11.2 × ln [INR] + 9.57 × ln[serum creatinine (mg / dL)] + 6.43

The result is multiplied by 10 and rounded to the nearest whole number. The value ranges from 6 to 40, in direct proportion to the severity of the disease.

The MELD model includes the impact of the variations of the evaluation of the components analysis in the laboratory, a lack of recognition of the differences between the creatinine in male and female and a potential disadvantage in certain groups of receptors, such as those with hyponatremia or those with primary sclerosing cholangitis[1718]. Encephalopathy, tumors and metabolic diseases are risk factors not correlated with the MELD, and it would be quite difficult to determine the priority of a patient with cirrhosis related complications and a low MELD score[19]. MELD is used in the age of patients older than 12 years.

The best way to use of MELD in the OLT is as an index to prioritize the patients on the waiting list and obtain a fair distribution of the organs obtained. A Child-Pugh score greater than 7 (stages B and C), or an MELD score greater than 15, and that is the indication for a transplantation.

However, there are circumstances in some patients in which survival decreases drastically, which are either should be deterioration of quality of life (like intractable pruritus), or because of a greater complication (such as ascites, encephalopathy, refractory digestive bleeding from portal hypertension, hepatorenal syndrome, spontaneous bacterial peritonitis).

In fact, the number of donor livers is not sufficient for the candidates of the recipient, and the number of patients on the waiting list is increasing, with the consequent increase in mortality on the waiting list. Some researchers suggest that the allocation of organ resources should be based primarily on the access of cadaveric donor organs to those recipients whose quality of life is unacceptable but without terminal disease, such as those with intractable pruritus, polycystic hepatis or intractable

encephalopathy[18]. It is necessary to choose well both the donors and the recipients, optimize the candidates and determine the appropriate time for the transplant, in order to obtain the best possible performance of the organs transplantation.

1.3. Surgical technique of liver transplantation

There are three steps of the surgical OLT of recipient: 1. Total hepatectomy; 2. Liver implant (vascular and biliary reconstruction); 3. Hemostasis and abdominal closure. The traditional technique "veno-venous bypass" was replaced by the technique "piggyback", which is not necessary to replace the vena cava, and make the hepatectomy with preservation of native retrohepatic vena cava. The ‘piggy-back’ technique with temporary portocaval shunt allows one to choose the order of graft revascularization. We prefer to do the portal anastomosis first and then the arterial anastomosis [20],[21].

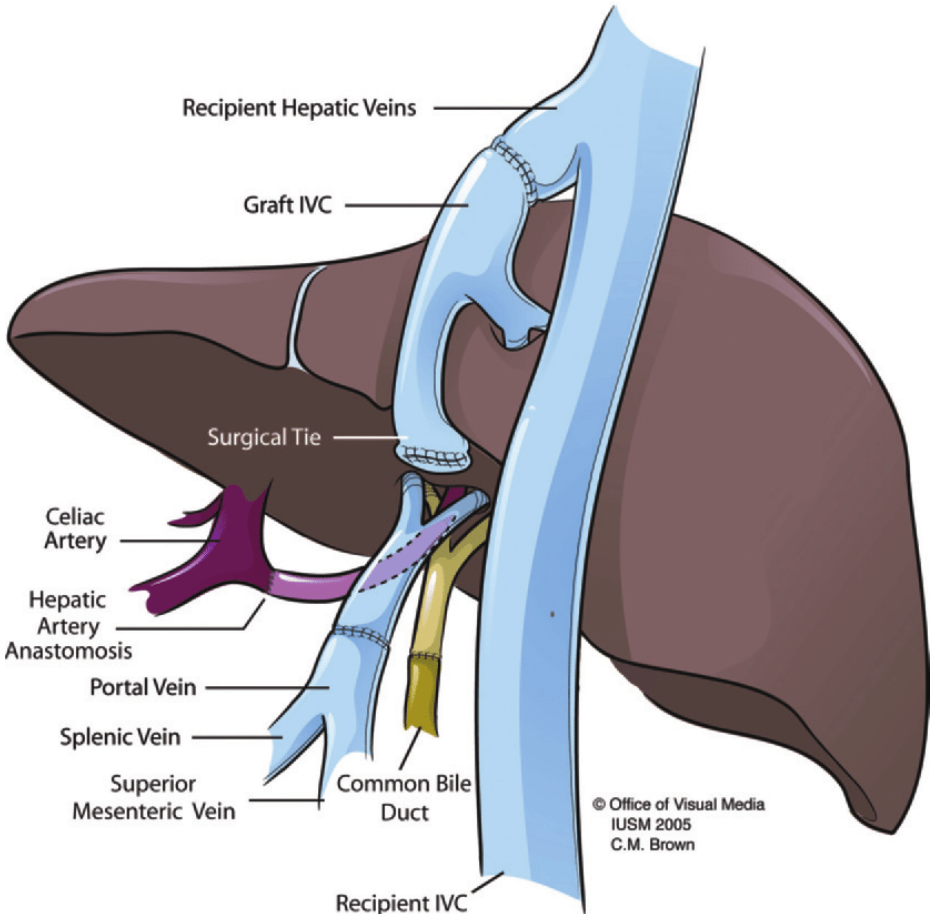


Figure 1.3. The piggyback technique for OLT. Diagram shows the confluence of donor hepatic veins that are anastomosed to the recipient suprahepatic IVC. End-to-end hepatic artery anastomosis, common bile duct anastomosis, and anastomosis of the donor and recipient portal veins are performed, although the exact technique may vary from case to case

The "piggyback" technique of liver transplantation had been described by Calne et al. in 1968 and had been routine used in clinical by Tzakis 20 years later[22]. The character is keeping the continuity of the vena cava of the recipient to implant in it a segment of the vena cava of the graft that contains the ostia of the hepatic veins. The technique offers the advantage of this technique are that they contain a reduction in the risk of stenosis, technical failures associated with the fabrication of two anastomoses with IVC, both reduction in the time of operation and bleeding[23].

In the technique, the suprahepatic veins are sectioned intrahepatic, in order to gain the largest possible segment of vein to anastomosis. In general, the common trunk of the middle and left hepatic veins are very short, so for the anastomosis of the

suprahepatic cava easier, the anastomosis area of the vena cava must be prepared. During the liver removing, The impermeability of the stump of the infrahepatic cava is ensured by obliterating the implantation holes of the caudate lobe veins, sectioned[22,24,25].

1.4. Complication of liver transplantation

Liver transplantation (LT) is widely accepted as an effective therapeutic modality for a variety of irreversible acute and chronic liver disease. But for the patient follow-up with OLT, there are several complication frequent in postoperative immediately and the long-term. As we know, LT is the treatment complicated, any complication is related to the mortality of the OLT[26].(Tab.1.3)

Immediate complications

- Medical complications
  - Hemodynamic complications
  - Respiratory changes
  - Renal dysfunction
  - Neurological complications
- Technical complications
  - Postoperative hemorrhage
  - Vascular complications
  - Biliary tract complications
- Liver graft dysfunction
  - Primary poor function
  - Acute cellular rejection
  - Recurrent viral hepatitis
- Infections
  - Bacterial
  - Viral
  - Fungal

Long-term complications

- Chronic rejection
- Renal failure
- Arterial hypertension
- Diabetes mellitus
- Dyslipidemia
- Obesity
- Bone complications
- Neurological complications
- Malignancy

Tab.1.3 Complication of liver transplantation

1.4.1. Complication postoperative immediately

Immediate postoperative complication

- *Postoperative hemorrhage*

It happens mainly during the first 24-48 h after completion of the OLT, with a variable prevalence that, in some series, has reached 20%[26]. Preexisting coagulopathy, significant hemorrhage during surgery, imperfect hemostasis and/or

immediate poor synthetic function are some of the factors associated with this complication. It has been considered a complication and a major problem, and due to its severity, up to 12% of the causes of post-transplant mortality have been reached in some series[2627].

- *Liver graft dysfunction*

The transplanted liver can have a normal postoperative course, manifested by progressive decrease of transaminases, increase of factor V, prothrombin and platelets, control of acidosis, normalization of ammonium, good biliary production, and absence of encephalopathy. Dysfunction of the graft may occur in the immediate postoperative period or late during the follow-up of the patient. The reason of early dysfunction of the graft can be: 1. problems of the graft itself (primary dysfunction/malfunction, nonspecific cholestatic syndrome, rejection), 2. complications of the surgical technique [vascular (arterial, portal thrombosis, poor drainage of the suprahepatic veins), or biliary], and 3. other causes such as drugrelated liver toxicity. Primary graft failure is defined as the clinical situation in which there is poor liver function to maintain the individual's life leading to death of the patient or retransplantation during the first seven postoperative days. It constitutes between 20 and 30% of the causes of retransplantation. The mortality of the primary dysfunction of graft without retransplantation approaches 80% of the patients, reducing between 30% and 50% after performing the retransplant[2628]. It is one of the most serious situations in the early post-transplant setting. The exact cause of this severe complication is unknown, although there are some of factors conditions: donor age, hemodynamic instability, suboptimal donors, cold ischemia time, reperfusion damage and the temperature of the preservation solution[262729].

- *Arterial complication*

This complications including thrombosis and stenosis of the hepatic artery, which cause high morbidity and mortality when they cause severe graft dysfunction. Particularly the thrombosis of the hepatic artery (prevalence ranging from 1.5 to 25%) are the most frequent ones. The most frequent cause including poor arterial flow, increased sinusoidal resistance, preservation injury, stenosis of the anastomosis and a state of hypercoagulability[26273031]. It usually manifests as a sudden and progressive deterioration of liver function, unlike primary graft dysfunction, it occurs after a variable period of normal hepatic function.

- *Portal vein complication*

Portal vein thrombosis is an infrequent complication with an overall prevalence of 2-3%. It is related to pre-transplantation portal thrombosis, splenectomy, and prior portal hypertension surgery. The most frequent cause are technical errors related to venous redundancy and torsion and / or stenosis of the anastomosis. Clinically, it has been classified as early form(in the first week of the postoperative period) and late form(as of the first week). The clinical picture is dominated by symptoms/signs of hepatic failure in the early form; and in the late form, the typical presentation was manifested portal hypertension, obviously, it is also accompanied by ascites, esophagogastric varices and other symptoms[2631].

- *Caval vein complication*

It is uncommon and rare complication following liver transplantation “thrombosis of the vena cava” with an reported incidence of less than 3%. The main risk factor leading to caval anastomosis complications is represented by technical errors in the connection of caval anastomoses, and usually realized with stenosis anastomosis. Clinical presentation ranges from hepatomegaly, ascites, Budd-Chiari syndrome, lower limb edema, liver and renal failure to hypotension leading to allograft loss and multiorgan failure[31].

- *Biliary complication*

Anastomotic leaks and Biliary strictures stenosis are the very frequency in Biliary complication after liver transplantation. With an overall incidence of 5% to 25%[32]. The most common is biliary strictures or called biliary stenosis. Biliary strictures usually can be divided into two categories and they are anastomotic strictures and non-anastomotic strictures[3233]. Anastomotic strictures usually involve the anastomotic site, is more commonly associated with biliary reconstruction by hepaticojejunostomy and the main cause responsible can be inadequate anastomoses. The mechanism of non-anastomotic strictures remains unclear, but it is often related to ischemic events. It has a worse prognosis and a significant proportion of patients who require re-transplantation[3334].

As we know, the rejection after liver transplantation can be divided acute rejection and chronic rejection. Due to chronic rejection belongs to the complication long-term, it will be introduce sooner.

- *Acute rejection*

The acute cellular rejection (ACR) as the frequency rejection after the liver transplantation, and showed incidence of 10-30% in recipients during the first month after liver transplantation[35]. ACR is generally associated with the high-level of hepatic enzymes (such as: serum aminotransferases, alkaline phosphatase, gamma-glutamyl transpeptidase) and/or bilirubin, especially the gamma-glutamyl transpeptidase (GGT). Moreover, for the younger patient (age$<$40 years) and the recipient of LT without renal dysfunction, have the ACR with high possible. But it just can be achieved only with histologic analysis of a liver biopsy[353637].

- *Infection complication*

Infections continue to be one of the main complications that can contribute to the recipient's death during the first year[26]. There are series source of the infecting, such as the donor organ, transfused blood products and the reactivation of previous infection et.al. The most common is the bacterial infections. For the viral infections, the source of infecting are cytomegalovirus, Epstein-Barr virus, herpesvirus, hepatitis-B and hepatitis-C virus. Without doubt, Fungal also is one of the source of infecting. But the incidence of fungal is not high, most of these episodes were caused by Candida or Aspergillus[3839].

- *Respiratory complication*

The lung is one mainly organ, in order of frequency, that presents greater infectious problems in the immediate postoperative period of OLT. The complications such as pneumonia and acute respiratory distress syndrome (ARDS) are associated with higher mortality rates despite advanced diagnosis and treatment options. Among those with pneumonia and acute respiratory failure, mortality rates were 43.75% and 50%, respectively[40]. Pulmonary effusion also occurs frequently with the mortality rates were 35.7%, in most cases, caused by intraoperative diaphragmatic manipulation. Pulmonary effusion has associated with the higher MELD scores. On the other hand, Several preoperative factors combined with a high MELD increase the incidence of respiratory failure postoperative, such factors as: perioperative blood transfusion, fluid retention, severe restrictive lung patterns, and muscle atrophy related to poor nutritional status[41].

- *Neurological complication*

The neurological complications in the OLT are frequent, during the postoperative period varies widely from 10 to 42%. The most common CNS complications are confusion, seizures, posterior leuco-encephalopathy syndrome and the neurotoxic

side-effects of immunosuppressive drugs. Neurological complications are a significant cause of morbidity and mortality in patients who undergo liver transplantation, which in some series reach a mortality rate is close to 50%. They may happened immediately after the operation of OLT or later (a month, three o four months)[424344].

Long-term complication

- *Chronic rejection*

Pathogenesis of CR is not well characterized. The pathogenesis of CR is multifactorial and includes vascular occlusion, antibodies and cell mediated pathways. Pathophysiology of CR is not entirely clear but immune mechanisms are involved as changes of CR does not appear in isografts and sometimes it is extension and result of ACR[35]. With the improvement in immunosuppression regimen, it has been achieved to reduce the frequency of episodes of acute rejection, as well as the consequent beneficial effect on CR control. In some series, chronic rejection currently occurs in less than 5% of transplants[45].

- *Renal dysfunction*

Renal dysfunction (acute o chronic) is one important complication after LT, and it occurs in 17-95% of patients[46]. The alteration of renal function can affect directly morbidity and mortality. There are approximate 10-20% patients after LT occur renal dysfunction in the medium or long-term. It is close to 20% that the patients occur chronic renal dysfunction after OLT 5-10 years[47]. Some research present many preoperative factors may favour the occurrence of renal dysfunction after OLT, such as preoperative renal dysfunction, hepatorenal syndrome (HRS), pre-OLT low serum

albumin level, hypovolemia, ascites, concomitant chronic diseases leading to kidney injury (diabetes mellitus, hypertension), hepatitis C, Child-Pugh score, Meld score and the use of Calcineurin inhibitors (CNI)[46].

- *Arterial hypertension*

Arterial hypertension (AHT) is a frequent complication in liver transplant recipients. The incidence is between 50-70% in the first months post-transplantation but decreases thereafter probably due to the reduction of the immunosuppressive doses[26]. One of the most important factors in OLT is the administration of CNI. Steroids also play an important role and their withdrawal is associated with improved blood pressure. The incidence of hypertension with tacrolimus treatment is lower and later than cyclosporine. It seems that the intensity of vasoconstrictive action mechanisms and / or renal retention of tacrolimus sodium is lower than that of ciclosporin[2648].

- *Diabetes Mellitus*

A variable percentage of patients, 10-33% will develop diabetes mellitus following transplantation (de novo DM). Some patients, de novo DM resolves a few months after the transplantation, But in other hand, it will be permanent. The prevalence depends on the time elapsed since transplantation and particularly on the immunosuppressive drugs[2649].

- *Malignancy*

De *novo* malignancy developing after transplantation constitutes a well-known complication of organ transplantation, 5-15% of patients who receive a solid organ transplant develop a de novo tumor, with a prevalence of cancer being between 3-16%, double times higher than the normal population[5051]. It has been considered second cause of death in the long-term of patients after liver transplantation[52]. The cumulative

incidence rate for all de novo malignancies was 1.3% at 1 year, 7.2% at 3 years, 11.7% at 5 years, 17.9% at 8 years and 24.8% at 10 years[53].

- *Cardiovascular complications*

Cardiovascular diseases have become the main cause of mortality after OLT. Early cardiac complications are relatively frequent, and have been related with patients who have cardiovascular disorders in pre-transplantation, such as coronary artery disease (CAD), cirrhotic cardiomyopathy (CCM), and structural heart disease[54]. The factors of risk post-transplantation include advanced age, use CNI and undetected coronary disease.

1.5. BASIC NOTIONS OF ONCOLOGY

Liver cancer, also known as hepatic cancer and primary hepatic cancer, is cancer that starts in the liver. The most common liver cancer, accounting for approximately 90% of all primary liver cancers, is hepatocellular carcinoma (HCC), and is the most common cause of death in people with cirrhosis.

1.5.1. Hepatocellular carcinoma (HCC)

HCC is now the third leading cause of cancer deaths worldwide, and the morbidity is increasing every year. Ranges from 500,000 to 1 million and with a number of deaths between 600,000 and 700,000 per year[55]. The incidence of HCC is highest in Asia and Africa, intermediate in Japan and European countries in the Mediterranean area, and lower in northern Europe and the USA. That means there are differences risk factors and pathogenesis among the different countries.

HCC related with cirrhosis of liver, it mostly occur in cirrhosis patients. So, the risk factors include factors which cause chronic liver disease that may lead to cirrhosis.

There are many factors well- known to lead cirrhosis or / and hepatocellular carcinoma such as: Chronic viral hepatitis (Viral hepatitis B and C), Alcoholic ingestion, Aflatoxin, Iron overload state, Diabetes mellitus, Wilson's disease, Hemophilia[56].

The diagnosis of suspicion is usually made by imaging tests. The objective of these tests is the screening of high risk patients, the identification of small lesions, the differential diagnosis between space occupying lesions and the selection of the appropriate treatment for each patient. If want to know the development and the situation of HCC, Biopsy has been necessary.

Staging looks at the size of the cancer (tumour) and whether it has spread anywhere else in the body. There are different staging systems doctors can use for liver cancer. The TNM staging system is one of these. TNM stands for Tumour (the size of the primary tumour), Node (whether the cancer has spread to the lymph nodes), Metastasis(whether the cancer has spread to another part of the body).

Stage T normally from 1 to 4, however, T_x and T_0 have special significant:

T_x: Primary tumor cannot be assessed

T_0: No evidence of primary tumor

T_1: Solitary tumor ≤ 2 cm or > 2 cm without vascular invasion

T_{1a}: Solitary tumor ≤ 2 cm (with or without vascular invasion)

T_{1b}: Solitary tumor > 2 cm without vascular invasion

T_2: Solitary tumor > 2 cm with vascular invasion or multiple tumors, none >. 5 cm

T_3: Multiple tumors, at least one of which is > 5 cm

T_4: Tumor involves a major branch of the portal vein or hepatic vein or tumor directly invades adjacent organs other than the gallbladder or tumor perforates the visceral peritoneum

Stage N are from X to 1:

N_X: Regional lymph nodes cannot be assessed

N_0: No regional lymph node metastasis

N_1: Regional lymph node metastasis

Stage M:

M_0: No distant metastasis

M_1: Distant metastasis

According to stage TNM, there is a other stage based on TNM. Clinically, usually use the stages above mentions.(Tab. 1.4)

Stage IA	T1a	N0	M0
Stage IB	T1b	No	M0
Stage II	T2	N0	M0
Stage IIIA	T3	N0	M0
Stage IIIB	T4	N0	M0
Stage IVA	Any T	N1	M0
Stage IVB	Any T	Any N	M1

Tab. 1.4: Stage I-IV of hepatocellular carcinoma

Unfortunately, the diagnosis of HCC is often made with advanced disease when patients have become symptomatic and have some degree of liver impairment[56]. Treatment of hepatocellular carcinoma varies by the stage of disease, a person's likelihood to tolerate surgery, and availability of liver transplant:

Partial hepatectomy: when the cancer is limited in one or more tumour in the same segment of liver, surgically removing the malignant cells may be curative. This may be accomplished by resection the affected portion of the liver or in some cases by orthotopic liver transplantation of the entire organ.

"Downstaging" therapy: for the patient with moderately advanced disease and limited in liver. But the disease is too advanced to qualify for partial hepatectomy. The patient may be treated by targeted therapies in order to reduce the size or number of active tumors, with the goal for satisfy conditions of liver transplant after this treatment.

Liver transplantation: for limited disease which qualifies for potential liver transplantation, the patient satisfy the conditions of Lt and in the waiting list of LT.

Palliative treatment: for end-stage of disease, including spread of cancer beyond the liver or in persons who may not tolerate surgery, the objective of treatment decrease symptoms of disease and prolong the time of survival.

Loco-regional therapy refers to any one of several minimally-invasive treatment techniques to focally target HCC within the liver. These procedures are alternatives to surgery, and may be considered in combination with other strategies, such as a later liver transplantation[5758].

In the year 2004-05, the media of survival rate is 47-49%, with the development of diagnostic and the technique, the survival rate of patients who receive the treatment increased Although changes in survival rates for cases with distant HCC were less pronounced, patients reporting treatment had higher survival rates[59].

1.5.2. Lung cancer

Lung cancer, also named lung carcinoma, is a malignant lung tumor characterized by uncontrolled cell growth in tissues of the lung. These abnormal cells do not carry out the functions of normal lung cells and do not develop into healthy lung tissue and this growth can spread beyond the lung by the process of metastasis into nearby tissue or other parts of the body. As they grow, the abnormal cells can form tumors and interfere with the functioning of the lung, which provides oxygen to the body via the blood.

There are several factors caused lung cancer, such as smoking (Tobacco, Marihuana and passive smoking), Radon gas, Asbestos, Air pollution, Genetics and others causes (ionizing radiantion, toxic gases et. al.).

The mainly way to diagnosis lung cancer is chest radiograph, obviously, there are some others technique can help us to discriminate lung cancer, like CT image and biopsy. Same to HCC, in the TNM stage, T means size of primary tumor, N means node and M means metastasis to other organ[60].

Stage TNM:

T_x : Tumor cells present in sputum or bronchial washing, but tumor not seen with imaging or bronchoscopy

T_0 : No evidence of primary tumor

T_{is} : Carcinoma in situ

T_1 : Tumor size less than or equal to 3 cm across, surrounded by lung or visceral pleura, without invasion proximal to the lobar bronchus

T_{1mi} : Minimally invasive adenocarcinoma

T_{1a} : Tumor size ≤ 1 cm

T_{1b} : Tumor size > 1 cm, but ≤ 2 cm

T_{1c} : Tumor size > 2 cm but ≤ 3 cm

T_2 : Any of: Tumor size> 3 cm but ≤ 5 cm; Involvement of the main bronchus but not the carina; Invasion of visceral pleura; Atelectasis or obstructive pneumonitis extending to the hilum

T_{2a} : Tumor size > 3 cm but ≤ 4 cm

T_{2b} : Tumor size > 4 cm but ≤ 5 cm

T_3 : Any of: Tumor size > 5 cm but ≤ 7 cm; Invasion into the chest wall, phrenic nerve, or parietal pericardium; Separate tumor nodule in the same lobe

T_4 : Any of: Tumor size > 7 cm; Invasion of the diaphragm, mediastinum, heart, great vessels, trachea, carina, recurrent laryngeal nerve, esophagus, or vertebral body or Separate tumor nodule in a different lobe of the same lung

N_X : Regional lymph nodes cannot be assessed

N_0 : No regional lymph node metastasis

N_1 : Metastasis to ipsilateral peribronchial and/or hilar lymph nodes

N_{1a} : Metastasis to a single N1 nodal station

N_{1b} : Metastasis to two or more N1 nodal stations

N_2 : Metastasis to ipsilateral mediastinal and/or subcarinal lymph nodes

N_{2a1} : Metastasis to one N2 nodal station with no involvement of N1 nodes

N_{2a2} : Metastasis to one N2 nodal station and at least one N1 nodal station

N_{2b} : Metastasis to two or more N2 nodal stations

N_3 : Any of : Metastasis to scalene or supraclavicular lymph nodes or Metastasis to contralateral hilar or mediastinal lymph nodes

M_X : Distant metastasis cannot be assessed

M_0 : No distant metastasis

M_{1a} : Any of : Separate tumor nodule in the other lung, Tumor with pleural or pericardial nodules or Malignant pleural or pericardial effusion

M_{1b}: A single metastasis outside the chest

M_{1c}: Two or more metastases outside the chest

	N0	N1	N2	N3
T1	IA	IIB	IIIA	IIIB
T2a	IB	IIB	IIIA	IIIB
T2b	IIA	IIB	IIIA	IIIB
T3	IIB	IIIA	IIIB	IIIC
T4	IIIA	IIIA	IIIB	IIIC
M1a	IVA	IVA	IVA	IVA
M1b	IVA	IVA	IVA	IVA
M1c	IVB	IVB	IVB	IVB

Tab. 1.5: Stage I-IV of lung cancer

Treatment for lung cancer depends on the cancer's specific cell type, how far it has spread, and the person's performance status. Common treatments include palliative care, surgery, chemotherapy, and radiation therapy.

The lung cancer five-year survival rate is 18.6% and the five-year survival rate for lung cancer is 56% for cases detected when the disease is still localized (within the lungs). Only 16% of lung cancer cases are diagnosed at an early stage. For distant tumors (spread to other organs) the five-year survival rate is only 5%. More than half of people with lung cancer die within one year of being diagnosed.

1.5.3. Colorectal cancer

Colorectal cancer (CRC) is the development of cancer from the colon or rectum (parts of the large intestine). A cancer is the abnormal growth of cells that have the ability to invade or spread to other parts of the body.

More than 75–95% of colorectal cancer occurs in people with little or no genetic risk. Risk factors include advanced age, male sex, high intake of fat, sugar, alcohol, red meat, processed meats, obesity, smoking, and a lack of physical exercise[6162].

Colorectal cancer diagnosis is performed by sampling of areas of the colon suspicious for possible tumor development, typically during colonoscopy or sigmoidoscopy, depending on the location of the lesion[61]. It is confirmed by microscopical examination of a tissue sample.

Stage is typically made according to the TNM staging system from the WHO organization.

T_X : The primary tumor cannot be evaluated.

T_0 : No evidence of cancer in the colon or rectum.

T_{is} : carcinoma in situ (also called cancer in situ). Cancer cells are found only in the epithelium or lamina propria, which are the top layers lining the inside of the colon or rectum.

T_1 : The tumor has grown into the submucosa, which is the layer of tissue underneath the mucosa or lining of the colon.

T_2 : The tumor has grown into the muscularis propria, a deeper, thick layer of muscle that contracts to force along the contents of the intestines.

T_3 : The tumor has grown through the muscularis propria and into the subserosa, which is a thin layer of connective tissue beneath the outer layer of some parts of the large intestine, or it has grown into tissues surrounding the colon or rectum.

T_{4a} : The tumor has grown into the surface of the visceral peritoneum, which means it has grown through all layers of the colon.

T_{4b} : The tumor has grown into or has attached to other organs or structures.

N_X : The regional lymph nodes cannot be evaluated.

N_0 : No spread to regional lymph nodes.

N_{1a} : There are tumor cells found in 1 regional lymph node.

N_{1b} : There are tumor cells found in 2 or 3 regional lymph nodes.

N_{1c} : There are nodules made up of tumor cells found in the structures near the colon that do not appear to be lymph nodes.

N_{2a} : There are tumor cells found in 4 to 6 regional lymph nodes.

N_{2b} : There are tumor cells found in 7 or more regional lymph nodes.

M_0 : The disease has not spread to a distant part of the body.

M_{1a} : The cancer has spread to 1 other part of the body beyond the colon or rectum.

M_{1b} : The cancer has spread to more than 1 part of the body other than the colon or rectum.

M_{1c} : The cancer has spread to the peritoneal surface.

Stage I-IV of colorectal cancer is complicated. (Tab. 1.6)

Stage I	T_1 or T_2	N_0	M_0
Stage IIA	T_3	N_0	M_0
Stage IIB	T_{4a}	N_0	M_0
Stage IIC	T_{4b}	N_0	M_0
Stage IIIA	T_1 or T_2	N_1 or N_{1c}	M_0
	T_1	N_{2a}	M_0
Stage IIIB	T_3 or T_{4a}	N_1 or N_{1c}	M_0
	T_2 or T_3	N_{2a}	M_0
Stage IIIC	T_{4a}	N_{2a}	M_0
	T_3 or T_{4a}	N_{2b}	M_0
	T_{4b}	N_1 or N_2	M_0
Stage IVA	Any T	Any N	M_{1a}
Stage IVB	Any T	Any N	M_{1b}
Stage IVC	Any T	Any N	M_{1c}

Tab. 1.6: Stage I-IV of colorectal cancer

In general, the treatment of colorectal cancer is cure or palliation. However, the decision on which aim to adopt depends on factors, including the health situation of patient, preferences and the stage of the tumor. When colorectal cancer is early stage, surgery can be curative. However, when it is detected at later stages (metastases are present), the treatment is usually directed at palliation, to relieve symptoms caused by the tumor and keep the person as comfortable as possible[6163].

According stage TNM, without N or M(N_0 and M_0): The colon cancer five-year survival rate is 90% and the five-year survival rate for rectal cancer is 89%; without M or written M_0: The colon cancer five-year survival rate is 71% and the five-year survival rate for rectal cancer is 70%; once the situation of tumor reach to late stage (M_1), the five-year survival rate reduce to 14%(colon cancer) and 15%(rectal cancer)[64].

1.5.4. Kidney cancer

The kidney cancer, or named renal cancer is the abnormal cell of kidney growth without limited, and affect renal function. Commonly, the causes of kidney cancer include smoking, obesity, faulty genes, family history with kidney cancer, with kidney disease and need dialysis, being infected with hepatitis C and previous treatment for testicular cancer or cervical cancer. Obviously, there are other cause, like kidney stone and artery hypertension et. al. that are researching.

According the pathophysiology and the tumor location, kidney cancer can be divide a several type. The most commonly types are renal cell carcinoma (RCC) and transitional cell carcinoma (TCC, also named urothelial carcinoma). Normally, blood and urine tests, biopsy and imagen tests (CT, X-Ray, MRI, IVP and Cystoscopy and nephro-ureteroscopy) are the way to diagnosis kidney cancer. In additional, the different type of kidney cancer develop with different way, that means there are different long-term outcomes. So, needs to make different treatment depend the stage and the type[65].

Stage TNM

T_X : The primary tumor cannot be evaluated.

T_0 : No evidence of a primary tumor.

T_1 : The tumor is found only in the kidney and ≤ 7 cm at its largest area. There has been much discussion among doctors about whether this classification should only include a tumor that ≤ 5 cm.

T_{1a} : The tumor is found only in the kidney and ≤ 4 cm at its largest area.

T_{1b} : The tumor is found only in the kidney and 4-7 cm at its largest area.

T_2 : The tumor is found only in the kidney and > 7 cm at its largest area.

T_{2a} : The tumor is only in the kidney and is > 7 cm but ≤ 10 cm at its largest area.

T_{2b} : The tumor is only in the kidney and is > 10 cm at its largest area.

T_3 : The tumor has grown into major veins within the kidney or perinephric tissue, which is the connective, fatty tissue around the kidneys. However, it has not grown into the adrenal gland on the same side of the body as the tumor. The adrenal glands are located on top of each kidney and produce hormones and adrenaline to help control heart rate, blood pressure, and other bodily functions. In addition, the tumor has not spread beyond Gerota's fascia, an envelope of tissue that surrounds the kidney.

T_{3a} : The tumor has spread to the large vein leading out of the kidney, called the renal vein, or the branches of the renal vein; the fat surrounding and/or inside the kidney; or the pelvis and calyces of the kidney, which collect urine before sending it to the bladder. The tumor has not grown beyond Gerota's fascia.

T_{3b} : The tumor has grown into the large vein that drains into the heart, called the inferior vena cava, below the diaphragm. The diaphragm is the muscle under the lungs that helps breathing.

T_{3c} : The tumor has spread to the vena cava above the diaphragm and into the right atrium of the heart or to the walls of the vena cava.

T_4 : The tumor has spread to areas beyond Gerota's fascia and extends into the adrenal gland on the same side of the body as the tumor.

N_X : The regional lymph nodes cannot be evaluated.

N_0 : The cancer has not spread to the regional lymph nodes.

N_1 : The cancer has spread to regional lymph nodes.

M_0 : The disease has not metastasized.

M_1 : The cancer has spread to other parts of the body beyond the kidney area.

Stage I	T_1	N_0	M_0
Stage II	T_2	N_0	M_0
Stage III	T_1 or T_2	N_1	M_0
	T_3	Any N	M_0
Stage IV	T_4	Any N	M_0
	Any T	Any N	M_1

Tab.1.7: Stage I-IV of kidney cancer

Surgery is the most common treatment as kidney cancer does not often respond to chemotherapy and radiotherapy. It usually be removed by surgery, if the cancer without metastasis. In some special cases, may remove the whole kidney. However most tumors are amenable to partial removal to eradicate the tumor and preserve the remaining normal portion of the kidney. If the cancer cannot be treated with surgery, other techniques such as freezing the tumor or treating it with high temperatures may be used. Certainly there are some new treatment opinion with the technique developed, like biological therapies and immunotherapy[66]. Although the new technique developed fast, but the survival rate of end-stage also lower (Tab.1.8).

The survival rate of five-years with stage

Stage I	81%
Stage II	74%
Stage III	53%
Stage IV	8%

Tab.1.8: Survival rate of kidney cancer

1.5.5. Prostate cancer

The prostate is a little-sized gland with peculiar of male, and the main function is making seminal fluid. With the men get older, prostate increase it's size and lead benign prostatic hypertrophy(BPH). BPH is a common in old male people and the symptoms likes the cancer. In fact, BPH is not related with prostate cancer.

The prostate cancer is not clear symptoms in early stage. However, there are several symptoms that are similar with BHP caused by prostate cancer, such as, frequent urination, nocturia, difficulty starting and maintaining a steady stream of urine, hematuria, and dysuria[67].

As known, obesity, age and family history are the major risk factors caused prostate cancer. The morbidity of prostate cancer is variational rate that is increasing with the age get older. It's uncommon to see a patient who has been diagnosis prostate cancer under 45 years old. The age of diagnosis is approximately 75 years old. The main method of diagnosis are digital rectal examination (DRE), cystoscopy, transrectal ultrasonography and tumor marker (PSA), but the most important method to diagnosis and confirmation the cancer is biopsy[68].

Obviously, for make the treatment regimen, know stage of cancer is necessary. Due to the DRE and PSA can make sure that the cancer is activity, there are several different stage to describe prostate cancer such as Stage TNM, Gleason score and Stage groping[69](Tab. 1.9).

Stage TNM

Clinical T

T_X: The primary tumor cannot be evaluated.

T_0: There is no evidence of a tumor in the prostate.

T_1: The tumor cannot be felt during a DRE and is not seen during imaging tests. It may be found when surgery is done for another reason, usually for BPH or an abnormal growth of noncancerous prostate cells.

T_{1a}: The tumor is in 5% or less of the prostate tissue removed during surgery.

T_{1b}: The tumor is in more than 5% of the prostate tissue removed during surgery.

T_{1c}: The tumor is found during a needle biopsy, usually because the patient has an elevated PSA level.

T_2: The tumor is found only in the prostate, not other parts of the body. It is large enough to be felt during a DRE.

T_{2a}: The tumor involves one-half of 1 side of the prostate.

T_{2b}: The tumor involves more than one-half of 1 side of the prostate but not both sides.

T_{2c}: The tumor has grown into both sides of the prostate.

T_3: The tumor has grown through the prostate on 1 side and into the tissue just outside the prostate.

T_{3a}: The tumor has grown through the prostate either on 1 or both sides of the prostate. This called extraprostatic extension (EPE).

T_{3b}: The tumor has grown into the seminal vesicle(s), the tube(s) that carry semen.

T_4: The tumor is fixed, or it is growing into nearby structures other than the seminal vesicles, such as the external sphincter, the part of the muscle layer that helps to control urination; the rectum; the bladder; levator muscles; or the pelvic wall.

Pathological T (there is not T_X, T_0 and T_1 in pathological T)

T_2: The tumor is found only in the prostate.

T_3: There is EPE. The tumor has grown through the prostate on 1 or both sides of the prostate.

T_{3a}: There is EPE or the tumor has invaded the neck of the bladder.

T_{3b}: The tumor has grown into the seminal vesicle(s).

T_4: The tumor is fixed, or it is growing into nearby structures other than the seminal vesicles, such as the external sphincter, the part of the muscle layer that helps to control urination; the rectum; the bladder; levator muscles; or the pelvic wall.

The stage N and M no present clinical or pathological.

N_X: The regional lymph nodes cannot be evaluated.

N_0: The cancer has not spread to the regional lymph nodes.

N_1: The cancer has spread to the regional (pelvic) lymph node(s).

M_X: Distant metastasis cannot be evaluated.

M_0: The disease has not metastasized.

M_1: There is distant metastasis.

M_{1a}: The cancer has spread to nonregional, or distant, lymph node(s).

M_{1b}: The cancer has spread to the bones.

M_{1c}: The cancer has spread to another part of the body, with or without spread to the bone.

Gleason score

Gleason X: The Gleason score cannot be determined.

Gleason 6 or lower: The cells are well differentiated, meaning they look similar to healthy cells.

Gleason 7: The cells are moderately differentiated, meaning they look somewhat similar to healthy cells.

Gleason 8, 9, or 10: The cells are poorly differentiated or undifferentiated, meaning they look very different from healthy cells.

Gleason scores are often grouped into simplified Grade Groups:

Grade Group 1 = Gleason 6

Grade Group 2 = Gleason 3 + 4 = 7

Grade Group 3 = Gleason 4 + 3 = 7

Gleason Group 4 = Gleason 8

Gleason Group 5 = Gleason 9 or 10

Stage I	cT1a-cT1c cT2a or pT2	N0 N0	M0 M0	PSA<10 Grade Group 1
Stage IIA	cT1a-cT1c cT2a-cT2c	N0 N0	M0 M0	PSA<20 Grade Group 1
Stage IIB	T1-T2	N0	M0	PSA<20 Grade Group 2
Stage IIC	T1-T2	N0	M0	PSA<20 Grade Group 3-4
Stage IIIA	T1-T2	N0	M0	PSA≥20 Grade Group 1-4
Stage IIIB	T3-T4	N0	M0	Any PSA Grade Group 1-4
Stage IIIC	Any T	N0	M0	Any PSA Grade Group 5
Stage IVA	Any T	N1	M0	Any PSA Any Grade Group
Stage IVB	Any T	N0	M1	Any PSA Any Grade Group

Tab. 1.9: Stage groping(I-IV) of prostate cancer

1.6. Immunosuppression in liver transplantation

The objective to make patient with liver transplantation intaking immunosuppressive drug is controlling and withstanding the acute rejection or chronic rejection. The rejection is a normal result because it is about genetic incompatibility between donor and recipient. Due to the donor's liver is an exotic organ for recipient, the immune system of recipient reacts to against the donor's histocompatibility antigens. Because recipient intake the immunosuppressive drug that reduces ability of immune, the risk of infection and development of tumors relative increase. So, one successful transplantation is not just finisher an operation, is find a balance between the rejection with graft can not work and the risk of infection increasing.

With the development of technique and pharmacology, there are kinds of drug can be selected. At same time, the immunobiology knowledge can show us the mechanisms of action of the drugs and the key point of the immune reaction[70].

The experience in the last 20 years makes us to know the immunosuppressive drug and the regimen must be adapted to each recipient. Depend the different factors(age, gender, race, indication of transplant, relative disease and general situation) of recipient to make the immunosuppression regimen individual. The aim of immunosuppressive therapy are not just for maintain the graft, and increase the quality of recipient's life

Certainly, the toxic effects of the different immunosuppressive drug such as Artery hypertension, Kidney failure, Diabetes mellitus, brain alterations et.al.) are allowed before the LT. If can reach a situation that the immunosuppressive drug has been withdrawn without reaction of rejection, that would be a liver transplantation successfully.

1.6.1. Calcineurin inhibitors

The calcineurin inhibitors bind to calcineurin, inhibiting calciumdependent activation of the synthesis and release of IL-2, and the result is the inhibition of IL-2 gene transcription and T cell activation and proliferation[71].

Now, CNIs are the basis of immunosuppressive treatment protocols after OLT, and cyclosporine-A (CyA) and tacrolimus are the two CNIs approved for use in organ transplantation. So, several studies have been compared the safety and efficacy between CyA and Tacrolimus to decide the drug of choice. In some studies, it seems tacrolimus is more powerful than CyA, especially in term to prevent acute rejection and improve the survival of recipient and graft. However, another more recent multi-center trial showed no significant differences between the two medications with regard to acute rejection episodes, death or graft loss[71].

As we known, CNIs have a wide range of toxicities such as, Nephrotoxicity and Neurotoxicity. Tacrolimus has a higher incidence of diabetes and tremor than CsA, on the other hand, CyA present the higher incidence of artery hypertension and gingival hyperplasia. For these reasons, we do not have objective data to decide which of them should be the better choice of CNI agents for all patients[7172].

1.6.1.1. Tacrolimus

Tacrolimus also named FK506, it is a 23-membered macrolide lactone that was first discovered in 1987 from the bacterium Streptomyces tsukubaensis. With a liposoluble nature and the mechanism of action similar to CyA, despite its different chemical structure and with a power of 100 times higher[73]. Compared the efficacy with CyA, the most research present there are not conspicuous different. In any case, although the long-term results do not show differences between CyA and tacrolimus, however, in the short-term it seems that the tacrolimus presents lower incidence of

acute rejection and cardiovascular risk, many units of transplants indicate to use tacrolimus[71]. The mechanism action of tacrolimus is similar with CyA, activating after binding to its intracellular receptor, an immunophilin called FK506 binding protein-12 (FKBP12). Like CyA, the FKBP-FK506 complex has no effect on the calcium-independent activation of T cells. The different point is that tacrolimus has the additional property of interfering with the expression of IL-4 receptors of B lymphocytes, of inhibiting the synthesis of IL-5 (B cell differentiation factor) to reducing expression of adhesion molecules in endothelial cells and block the response of leukocytes to IL-8[7475].

Oral tacrolimus is slowly absorbed in the tract of duodenum and jejunum without depending on bile production. After intake 1.5-3 hours, the highest maximum concentrations (Cmax) reached. The total bioavailability is very low, just 20 to 25%. In the blood, tacrolimus is mainly bound to erythrocytes, especially albumin; more than 98.8% are bound to plasma proteins. The substance is metabolized in the liver, for this reason, liver failure produces an increase in their plasma levels. Biological half-life varies widely, due to differences in clearance. Tacrolimus is predominantly eliminated via the faeces in form of its metabolites. Less than 1% is actively eliminated by the kidneys or fecal route, so it does not need to modify its dose in case of renal failure (unless renal failure is attributed to the drug)[73].

1.6.1.2. Toxicity of calcineurin inhibitors

CNIs present multiple and relatively adverse side effects. The most frequent and related are nephrotoxicity, neurotoxicity and de *de novo* neoplasia.

- Nephrotoxicity

As described above, nephropathy is an important complication of post-LT, and the it is caused by used of CNI. The mechanism and action of CyA and tacrolimus are

similar, although with different structure. The incidence of nephrotoxicity due to CNI, although using low doses, is 20% present chronic renal failure within 5 years[71].

The CNIs cause to two different nephrotoxicity at high levels. The first is called acute nephrotoxicity associated with acute renal failure reversible and is characterized by a decrease in glomerular filtration and renal plasma flow that produce an increase in creatinine and urea. And induce renal dysfunction and also tubular injury caused CNIs afferent renal arteriolar vasoconstriction. This vasoconstrictor effect is dose related and reversible[70].

On the other hand, the chronic renal nephrotoxicity that occurs long-term after 6-12 months of the beginning of treatment and advanced renal failure in 8% to 28%[76]. The characterized by the development of chronic renal failure whose pathophysiology is characterized by the presence of structural lesions, such as fibrosis. interstitial and the appearance of renal vascular sclerosis, not being reversible despite decreasing the dose and in some cases progressing to end-stage renal failure and dialysis.

- *De novo* neoplasia

With the immunosuppressive agent used, the immune of humans reducing by time, so the *de novo* neoplasm is also a complication post-LT and associated relationally with CNI. The incidence of post-transplant cancer has a clear relationship with the time of exposure to immunosuppressive treatment. Cancer is currently one of the major limitations of the expectation and quality of life of the patient carrying a solid organ transplant. Experimental and clinical arguments support an association between the immunosuppressed state and cancer. CNI increases the expression of transforming-growth factor beta(TGF-β), which favors the appearance of several factors of an invasive phenotype of neoplastic cells, of the angiogenesis induce vascular endothelial growth factor and of interleukin-6, which increases Epstein-Barr virus-induced B-cell

growth.18 Moreover, cyclosporine interferes with DNA repair among in vitro cultured white blood cells from renal transplant recipients[76].

1.6.2. Mycophenolate-mofetil

Mycophenolate-mofetil (MMF) is an immunosuppressive agent used to prevent rejection in organ transplantation. Discovered by Italian medical scientist Bartolomeo Gosio in 1893, mycophenolic acid was the first antibiotic to be synthesised in pure and crystalline form. MMF was approved by the FDA in 1995.

Mechanism of action

MMF was the observations that suggested that the de novo pathway of purine synthesis, and not the recovery pathway, was crucial for the proliferative response of human T and B lymphocytes.

Mycophenolate mofetil (MMF) undergo immediate first-pass metabolism in the liver into the active compound mycophenolic acid (MPA). MPA inhibits inosine-5'-monophosphate dehydrogenase (IMPDH). IMPDH inhibition particularly affects lymphocytes since they rely almost exclusively on de novo purine synthesis.

Clinical use

In general, it has been used mainly associated with anti-calcineurin or as a drug to be added in case of toxicity to CyA or tacrolimus given the need to reduce the dose of these due to the side effects they cause[73].

It has also been observed that the use of MMF has a beneficial effect in the prevention of chronic graft nephropathy, which is in part independent of the reduction of the acute or chronic rejection rate.

Adverse effects

Because of the mechanism of action, it alters other types of dividing cells, so fundamentally its side effects include gastrointestinal disorders, spinal toxicity and to a lesser extent opportunistic infections and respiratory tract disorders.

First, the digestive disorders include diarrhea, nausea, vomiting and joint pain. These usually involve the suppression of treatment in only 5% of cases.

Opportunistic infections: All transplant patients are at the situation that increasing risk of opportunistic infections, the most common in patients treated with MMF (2 or 3 g / day, but patients may tolerate the drug better if started at 500mg twice a day or four times a day[73].) in OLT are fungal (mainly Candida) and viral (viremia / CMV disease and Herpes simplex).

Unlike other immunosuppressive agents used in the OLT, no clinically significant adverse effects have been found as a direct consequence of the use of MMF such as nephrotoxicity, hepatotoxicity.

1.6.3. mTORi

Sirolimus, or called rapamycin, is a macrolide compound, and it is produced by the bacterium Streptomyces hygroscopicus and was isolated for the first time in 1972 by Surendra Nath Sehgal and colleagues from samples of Streptomyces hygroscopicus

found on Easter Island[77], where it received its initial name. Sirolimus was initially developed as an antifungal agent in 1975, later demonstrating its immunosuppressive activity in vitro. However, this use was abandoned when it was discovered to have potent immunosuppressive and antiproliferative properties due to its ability to inhibit mTOR.

In recent years, there is other inhibitor was developed with the similar mechanism of sirolimus, called Everolimus that is the 40-O-(2-hydroxyethyl) derivative of sirolimus and works similarly to sirolimus as an inhibitor of mammalian target of rapamycin (mTOR).

Mechanism action of mTORi

This therapeutic group has also been called Proliferation Signal Inhibitors (ISP) and has some advantages over classical immunosuppressants that have aroused great interest. It acts through blocking signal 3 of cell activation from IL-2 receptors in T-cells and B-cells. Interestingly, despite binding to the same cell receptor, sirolimus and tacrolimus do not compete with each other and act synergistically[70]. The mTOR protein forms two structurally and functionally distinct complexes, mTORC1 and mTORC2, both of which play different roles downstream. mTORC1 is activated by growth factors, amino acids and controls of cell proliferation, promoting processes such as DNA translation, RNA transcription, ribosomal biogenesis, and cell cycle progression[7778].

Because the antiproliferative effects, it is useful in the artery of chronic rejection. In addition to blocking the cell cycle progression induced by IL-2, it facilitates apoptosis, acting synergistically with the blocking of the costimulatory signal in the reduction of alloreactive lymphocytes, which also differentiates it from anti-calcineurins, which block apoptosis. This is relevant because apoptosis has been related to the development of allograft tolerance mechanisms.

1.6.3.1. Pharmacokinetics of Sirolimus

Absorption

Sirolimus is absorbed quickly that means whole-blood sirolimus concentrations (C_{max}) occur 1 hour after administration of single doses and 2 hours after multiple doses in stable renal transplant patients, The bioavailability of sirolimus is 14%. Co-administration with fatty foods delays the rate of absorption, in this way the C_{max} decreases by 34%, the tmax increases 3.5 times, and the area under the curve of plasma concentrations vs. Time (AUC) is also increased by 35% when compared to the values obtained in fasting[79].

Metabolism

Sirolimus undergoes extensive oxidative metabolism at the liver level, after incubation with human liver ,icrosomes to yield multiple demethylation and hydroxylation reactions. It originates a large number of metabolites, and in appearance these partially preserve the activity of the original molecule[79].

Excretion

According to the result of the health volunteers intake labeled sirolimus with single oral dose, The excretion is mostly fecal (91.1%), with urinary elimination being low (2.2%). After the administration of multiple doses to kidney transplant patients, the elimination half-life (t1 / 2) was 62 hours and mean oral-dose clearance (Cl / F) 210 ml / h / kg[79].

1.6.3.2. Pharmacokinetics of Everolimus

Absorption

The oral bioavailability of everolimus is 16%, little higher than sirolimus. Everolimus reaches the maximum concentration after 1 or 2 hours after intake with oral administration. Based on the ratio of areas under the concentration / time curve (AUC ratio), the relative bioavailability of the dispersible tablet versus the conventional tablet is 0.90 (90% CI 0.76 - 1.07)[80].

Distribution

The blood / plasma rate of everolimus depends on the concentration. The binding to plasma proteins is approximately 74% in healthy individuals and in patients with moderate hepatic insufficiency.

Metabolism

Everolimus is one of the substrates of CYP3A4 and P-glycoprotein. The main metabolic pathways identified in humans are monohydroxylations and 0-dealkylations. Two major metabolites are formed by hydrolysis of the cyclic lactone. Everolimus is the predominant form in blood circulation. None of the major metabolites contribute significantly to the immunosuppressive activity of everolimus[81].

Excretion

Same to the experimentation of Sirolimus. After the administration of a single dose of radioactive everolimus to patients treated with ciclosporin after transplantation, most of the radioactivity (80%) was found in the faeces and only a small proportion (5%) in the urine[82]. And researcher undetected the original compound in urine and faeces.

1.6.3.3. Adverse effects

The main side effect is hyperlipidemia, present in approximately 44% of patients, a higher percentage than other immunosuppressive drugs. The presence of thrombopenia and leukopenia is also very frequent, although both are reversible in 90% of cases[83].

Some studies have shown a higher survival of the graft while others have described a relative myelosuppression. mTORi is regulated in eukaryotic cells by the PI3K / Akt metabolic pathway. Those cells with a mutation in the PTEN and PI3K genes present a release of the control of this pathway, so that the inhibition of mTOR would act effectively in the neoplastic control. It should be noted that there is an initial work that describes an increased risk of arterial thrombosis in patients who received sirolimus, although this finding has not been reproduced in other studies[82].

Use of these drugs in liver transplantation for malignancies (hepatocellular carcinoma, cholangiocarcinoma, neuroendocrine tumors metastatic), malignant tumors de novo (cutaneous carcinomas, gastric cancer, breast cancer, etc), renal failure associated with CNI, previous renal failure that does not improve after transplantation and immunosuppression in patients with acute or chronic cortico-resistant rejection[84].

The potential risks of mTOR inhibitors, including hypercholesterolemia, thrombosis and problems in wound healing, have also been highlighted in this population segment. No controlled study has examined these critical endpoints in the OLT setting.

1.6.3.4. mTORi in the field of oncology

The incidence of *de novo* neoplasia that occur after OLT is higher than that of malignant tumors developed in the general population, because of the recipient must administration immunosuppressive agent for lifelong[85].

Immunosuppressive therapy decreases the immune response against malignant cells and against a large variety of viruses with oncogenic properties. Likewise, it has been described in a recently published randomized trial that cirrhotic patients of enolic origin had a much higher risk of non-hepatic neoplasia in the case of having been transplanted, in contrast to those who did not receive a transplant and followed a treatment standard for their pathology (at 5 years, the risk of neoplasia was 37% versus 6%). This increased risk is especially high in neoplasms related to viral infections, such as non-Hodgkin's lymphoma, Kaposi's sarcoma and cervical cancer. The risk of skin cancer is also greatly increased in these patients[8687].

The incidence of other common cancers seems to be increased too, but this risk is not high. Some studies shown that the incidence of colorectal, lung, head and neck, urological, and hepatocellular carcinomas increases after receiving lifelong immunosuppressive therapy[888990]. In some cases, the cause of this increased risk may be a specific association between certain causes of liver disease and risk factors for the development of certain types of neoplasia in the general population: there is an association between primary sclerosing cholangitis and ulcerative colitis, which markedly increases the risk of colorectal cancer.

In patients with alcoholic liver disease, the risk of esophageal cancer and cancer of the head and neck is increased of tumors diagnosed most frequently in patients post-transplant. And it may have the association with high alcohol intaking and smoking, which is a very important risk factor for some patients.

mTOR, a protein of the group of kinases, is involved in cellular metabolic regulation PI3K / Akt. Its activation, in response to growth, nutrient and energy signals, leads to an increase in the synthesis of proteins necessary for the development and growth of tumors. This feature makes mTOR an important target for cancer therapy[77].

The first generation of mTORi, sirolimus and its derivatives, everolimus have been widely evaluated in patients with cancer. On the other hand, the second generation mTORi, small molecules with action in the field of kinases, are also in full clinical development. Clinical trials are under way to identify additional malignancies that respond to mTORi, either in single or combined therapy. Future research should evaluate the most appropriate terpeutic regimes as well as target populations.

Same to all immunosuppressive agents, the sirolimus also decreases the antioncogenic activity of the organism and allows the proliferation of some cancers which would normally be destroyed. Immunosuppressed patients have a cancer risk 10 to 100 times higher than the general population. In addition, patients who currently have or have been treated for cancer, develop a higher rate of tumor progression as well as recurrence in relation to patients with an intact immune system[909192].

1.6.3.4.1. Hepatocellular carcinoma recurrence

HCC is the most common in primary liver cancer with(70-85%), and it had been the third most frequent cause of death-related-cancer in the world[93]. As we know, liver transplantation is the ultimate treatment of end-stage HCC. Due to against reaction rejection of organ, from AZA to CNIs, and to mTORi, many regimens were be chosen for reduce the complications post-LT to lower. As mentioned above, mTORi has been used as anticancer drug.

The exact mechanism for this increased risk is unknown, although preclinical data suggest that CNIs may actually promote tumor growth. In contrast, other data suggest

that immunosuppression regimens that include rapamycin (sirolimus) or its analogs reduce the risk of HCC recurrence as well as the development of de novo malignancies after orthotopic liver transplantation, while successfully avoiding allograft rejection and improving overall survival[93].

The rate of HCC recurrence post-LT is between 13-27%. A meta-analysis indicated the risk factors on pre-transplant risk for HCC recurrence showed significant correlations for the presence of vascular invasion, level of differentiation, tumor size, and tumor stage outside the Milan criteria. As vascular invasion and a tumor size >5 cm are included in a tumor stage considered exceeding the Milan criteria, the only risk factor identified within this meta-analysis for patients with a tumor stage within the Milan criteria was a moderate or poorly differentiated HCC[94].

Theoretically, HCC recurrence can use all modalities for treating HCC. However, HCC recurrence after LT is considered a “systemic disease”, and the efficacy of locoregional treatment for a systemic disease is doubtful. Because for the recipient, use immunosuppressive agent can affect wound healing and increace the rate of infection. And the operation of liver transplantation may damage or change the structures of vascular in anatomy. Due to these reasons, it may be difficult in interventional radiological procedures like TACE. So, some studies present a new treatment for intrahepatic recurrent, High-intensity focused ultrasound (HIFU) ablation. And on the other hand, change the inhibitor non-mTORi to mTORi is a regimen can be used[95].

1.6.3.4.2. *De novo* neoplasia

Due to the more prolonged exposure to immunosuppression is associated with an increased frequency of developing neoplasms, the rate of De novo neoplasia almost reached 30% at 10 years in patient after liver transplantation and it is the most common cause to death in 1-year of recipient after liver transplantation. Compare with general

population, same level of the age and gender, there are 2-4 times higher of risk to occur malignancy[96].

De novo malignancy after liver transplantation has become a major source of morbidity and mortality[97]. As mentioned above, used inhibitor is the most important factor that can increase risk to occur malignancy. Obviously, there are the association factors with cancer risk such as: age, gender, race, alcohol intake, smoking and history of cancer et.al.[91]

Age: Advanced age as a risk factor for almost all disease, as the well described risk factor for *de novo* neoplasia after LT. This suggests that other factors may supersede age in cancer risk, though some caveats are notable with the extremes of age. A study reported, LT recipients older than 60 years had > 2 times higher 5-year incidence of new cancers (> 40%) compared to younger LT recipients (< 20%), with significantly higher cancer related mortality[91].

Gender and race: There is conflicting data on the relative risk of de novo malignancy according to gender, with slightly higher sirolimus of cancers in females in one registry study, and in males in another, limiting any meaningful conclusion. A study indicate that Non-Caucasian race was associated with a significantly increased risk of de novo malignancy, but the small size of that subgroup was limiting[9192].

Indication for LT: Patients who receive LT and conform to one of indications are more prone to occur malignancies. There are some research of multicenter in USA discovered that the patient with primary sclerosing cholangitis exhibited an increased risk for lymphoproliferative disorder (PTLD), skin malignancies and solid organ malignancies. And the highest cumulative incidence of non-skin cancer of 5.5%, 10.4%, and 21.9% at 1, 5, and 10 years, respectively.

Alcohol intake and smoke: Smoking is a significant predictor of *de novo* malignancies on analysis, and many studies have described the carcinogenic properties of alcohol and smoking in immunocompetent individuals. Alcoholic liver disease is associated with increased cancer risk post-LT. The synergy between the alcohol intake and smoking to affect carcinoma has been described[919298].

History of cancer: A history of cancer prior to LT was not associated with its recurrence after LT. However, LT for HCC has been associated with an increased risk of de novo malignancy, and we have mentioned above.

The risk of *de novo* malignancy is variable across a range of tumor types, and normally grouped three categories that including: skin cancers, lymphoproliferative disorder (PTLD) and solid organ cancers.

- Skin cancer

Skin cancer is the most common malignancy occurring in the situation after OLT and immunosuppression, except these, male sex, age older than 55 years and ultraviolet radiation also are the important risk factor in the pathogenesis of skin malignancies, and exerts a field cancerization mutagenic effect in exposed areas of the skin[91]. The incidence of skin cancer increases with extended survival after OLT. These cancer include squamous cell cancer (SCC), basal cell cancer (BCC) and melanomas, and the rate of SCC, BCC and melanomas are 50%, 40.9% and 9.1%, respectively[919699]. Malignant melanoma is the most serious form of skin cancer, and Early identification and surgical removal is currently the only proven therapy for melanoma[99].

Compare with general population, SCC is more common than BCC in recipient of transplantation. Additionally, while SCC and BCC are easily surveyed and resected, SCC can behave more aggressively in LT recipients[91].

- Lymphoproliferative disorder (PTLD)

Lymphoproliferative disorder (PTLD) is the second commonly malignancy in patient post-LT, and its widely distribution of age that even to the very young patient. PTLD encompasses a heterogeneous group of diseases characterized by excessive proliferation of lymphoid cells and it commonly caused by de novo infection, or reactivation of latent Epstein-Barr virus (EBV)[96]. Compared to other solid organ transplantation recipient, the rate of PTLD is lower in LT, that may due to lower immunosuppression levels needed to prevent liver allograft rejection, and possibly a smaller number of donor lymphocytes in the graft[91].

Some studies reported that the incidence frequency in pediatric patient is higher than adult, almost 3 fold. This is likely a reflection of the EBV negative status of pediatric recipient, whereas EBV infects 90% of the adults worldwide. The immunosuppressive regimen is very important for the recipient post-LT with PTLD, especially the use of anti-lymphocytic serum and tacrolimus in children. The indication for transplantation is also important, as the risk of PTLD in patients transplanted for sclerosing cholangitis is more than 20%; alcoholic cirrhosis and viral hepatitis are indications associated with an increased incidence of PTLD in adult recipients[100].

The diagnosis is based on tumor biopsy with molecular characterization of clonality. It is important to test the tumor for EBV, as the results have diagnostic, prognostic and therapeutic implications.

There are some therapy for PTLD such as, Withdrawal of immunosuppression, and surgery; Antiviral treatment; Chemotherapy and Immunotherapy (“Interferon-a and IL-

6", "Anti-B cell monoclonal antibodies" and "Adoptive immunotherapy with virus-specific T cells").

PTLD used to be highly lethal and 40–60% of solid organ recipients dead caused it. The prognosis has improved markedly with the use of new therapeutic approaches such as monoclonal antibodies and T-cell therapy. The overall survival rate of patients with PTLD is conservative, especially the rate of children is higher than in adults, with 65% of survival rate at 15 years in children compared to 39% for adults, and 55% at 10 years in adult and pediatric recipients with PTLD[100].

- Kaposi's sarcoma (KS)

KS is a multifocal angioproliferative mucocutaneous neoplasm driven by HHV-8 infection and represents approximately 4% of all post-transplant tumors. Compared with general population, the risk occurring neoplasia increasing 500 fold in patient who receive organ transplantation[96]. In some reported that the incidence of KS occurring recipient after LT is low, although the rate is higher than patient kidney transplantation (KT)[101].

Unlike others *de novo* neoplasia, the incidence of KS is reducing with the time after transplantation. Moreover, a study present the evidence to the usefulness of mTOR inhibitors in treating this tumor while at the same time providing effective immunosuppression[96102].

- Lung cancer

Obviously, the incidence of lung cancer increasing compared with general population. Expect long-term used immunosuppressive agent after transplantation, the risk factor of occur cancer almost as well as mentioned above. Especially smoking, as same as general population, its the most important factor to occur lung cancer.

According to the epitemiological related, in general, smoker always are heavy drinker, so the patients with alcohol-related cirrhosis as an indication for LT had higher rates of lung cancer than those who underwent LT for other indications[96].

For *novo* lung cancer after transplantation, the stage TNM and stage grouping are not different with mentioned above.

In principal, surgical is a good choice to solution. Pathological type of de novo lung cancer was adenocarcinoma and squamous cell carcinoma. Contraindications for surgery such as, cancer with malignant effusion; cancer with multiple pulmonary metastases or metastases distal; small cell lung cancer and others unresectable advanced cancer[103].

The survival rate of lung cancer *de novo* after LT is low, just 37.5% of 1-year.

- Colorectal cancer

The incidence of colorectal cancer (CRC) of patient post-LT increasing clearly compared with the general population. In general, some recipient preexisting risk factors for CRC such as primary sclerosing cholangitis (PSC) and associated inflammatory bowel disease (IBD), and these disease make an increasing risk of CRC[103104105].

The risk factors for increased incidence of de novo CRCs are considered to be age > 45 years, diagnosis of PSC, an intact colon, colonic polyps, and a longer duration of ulcerative colitis.

Due to the complication of post-transplantation, differential diagnoses in the post-transplantation period would be broader and include post-transplantation

complications such as rejection, intestinal ischemia, biliary complications, bowel obstructions caused by adhesions, internal hernias, and volvulus. Therefore, they require careful and extensive workup. Except CRC, the neoplastic bowel obstructions in this population might also be secondary to post-transplantation lymphoproliferative disease. As we know, diarrhea is a adverse effect of inhibitor immunosuppressive, confirm the cause of diarrhea also need a systemic approach like endoscopy and biopsy. And the CT might have an important role in the evaluation of suspected colorectal neoplasia and its potential metastatic diseases[105106].

The main regimen to treat colorectal cancer *de novo* are colectomy and chemotherapy o radiation therapy. Its depend the situation of patient and the stage of cancer to decide. A notice worthful is that the agents for chemotherapy need to have reasonable activity in the post–liver transplantation setting. However, further delineations of specific dosing, drug interactions with immunosuppressive agents, and safety on graft tolerance should be carefully[105].

More frequently diagnosed between 16 and 50 months after transplant, colorectal cancer in transplant recipients tends to be detected at an earlier age and has been associated with a worse prognosis compared to the general population. However, no data to shown the survival rate of LT recipient after diagnosed *novo* colorectal cancer[96].

- Oropharyngeal cancer

Neck neoplasms is more frequent in the LT recipient than in the general population, and mean time to diagnosis is reportedly between 34.3 months and 61.2 months[96].

Oropharyngeal cancer is 25.5 fold more frequent in patients transplanted for alcohol-related cirrhosis compared with transplanted for other indications, and the carcinogenic effects of smoking observed in the general population also applies for transplant recipients. In general, heavy smoker also tend to be heavy drinker. Due to

these reasons, smoke and alcohol are the important factor risk for oropharyngeal cancer[96].

Clinically, used the stage TNM and stage grouping (Tab. 1.10) to confirm regimen of treatment.

T:

T_X: The primary tumor cannot be evaluated.

T_{is}: Describes a stage called carcinoma (cancer) in situ. This is a very early cancer where cancer cells are found only in 1 layer of tissue.

T_1: The tumor is 2 centimeters (cm) or smaller at its greatest dimension.

T_2: The tumor is larger than 2 cm but not larger than 4 cm.

T_3: The tumor is larger than 4 cm or has spread to the epiglottis, which is the flap of cartilage that diverts food into the esophagus.

T_{4a}: The tumor has invaded the larynx, muscle of the tongue, muscles in the jaw, roof of the mouth, or jawbone.

T_{4b}: The tumor has invaded muscles and bones in the region of the mouth; the nasopharynx, which is the air passageway at the upper part of the throat behind the nose; or the base of the skull, or the tumor encases the carotid artery.

N:

N_X: The regional lymph nodes cannot be evaluated.

N_0: There is no evidence of cancer in the regional lymph nodes.

N_1: The cancer has spread to a single lymph node on the same side as the primary tumor, and the cancer found in the node is 3 cm or smaller. There is no ENE.

N_{2a}: Cancer has spread to a single lymph node on the same side as the primary tumor and is larger than 3 cm but not larger than 6 cm. There is no ENE.

N_{2b}: Cancer has spread to more than 1 lymph node on the same side as the primary tumor, and none measures larger than 6 cm. There is no ENE.

N_{2c}: Cancer has spread to more than 1 lymph node on either side of the body, and none measures larger than 6 cm. There is no ENE.

N_{3a}: The cancer is found in a lymph node and is larger than 6 cm. There is no ENE.

N_{3b}: There is ENE in any lymph node.

M:

M_0: Cancer has not spread to other parts of the body.

M_1: Cancer has spread to other parts of the body.

Stage 0	T_{is}	N_0	M_0
Stage I	T_1	N_0	M_0
Stage II	T_2	N_0	M_0
Stage III	T_3 T_1-T_3	N_0 N_1	M_0
Stage IVA	T_{4a} T_1-T_{4a}	N_0-N_1 N_2	M_0
Stage IVB	Any T T_{4b}	N_3 Any N	M_0
Stage IVC	Any T	Any N	M_1

Tab. 1.10: Stage Group (I-IV)

For the patient of LT, the diagnosis is same to general population. Usually used imagen test such as X-Ray, CT and MRI; endoscopy and biopsy also are the test diagnosis for oropharyngeal cancer[107]. Depend the stage, the regimen of therapy can be chosen surgical, radiation therapy o expectant therapy.

The survival rate of oropharyngeal cancer is not bad that the rate of 5-years is 66.67% and 10-years is 44.44%.[108]

- Esophageal and gastric cancer

Although their incidence is increased with respect to the general population, gastric and esophageal cancers are reported infrequently in most series of LT recipients[96]. Esophageal cancer following liver transplant is closely associated with history of alcohol intake and smoking chewing[109]. The factor risk of gastric cancer mainly is prolong time using immunosuppressive agent.

Stage TNM of gastric cancer

T_X: The primary tumor cannot be evaluated.

T_0: There is no evidence of a primary tumor in the stomach.

T_{is}: This stage describes a condition called carcinoma (cancer) in situ. The cancer is found only in cells on the surface of the inner lining of the stomach called the epithelium and has not spread to any other layers of the stomach.

T_1: The tumor has grown into the lamina propria, muscularis mucosae, or the submucosa, which are the inner layers of the wall of the stomach.

T_{1a}: The tumor has grown into the lamina propria or muscularis mucosae.

T_{1b}: The tumor has grown into the submucosa.

T_2: The tumor has grown into the muscularis propria, the muscle layer of the stomach.

T_3: The tumor has grown through all of the layers of the muscle into the connective tissue outside the stomach. It has not grown into the lining of the abdomen, called the peritoneal lining, or into the serosa, which is the outer layer of the stomach.

T_4: The tumor has grown through all of the layers of the muscle into the connective tissue outside the stomach. It has also grown into the peritoneal lining or serosa or the organs surrounding the stomach.

T_{4a}: The tumor has grown into the serosa.

T_{4b}: The tumor has grown into organs surrounding the stomach.

N_X: Regional lymph nodes cannot be evaluated.

N_0: The cancer has not spread to the regional lymph nodes.

N_1: The cancer has spread to 1 to 2 regional lymph nodes.

N_2: The cancer has spread to 3 to 6 regional lymph nodes.

N_3: The cancer has spread to 7 or more regional lymph nodes.

N_{3a}: The cancer has spread to 7 to 15 regional lymph nodes.

N_{3b}: The cancer has spread to 16 or more regional lymph nodes.

M_X: Distant metastasis cannot be evaluated.

M_0: The cancer has not spread to other parts of the body.

M_1: The cancer has spread to another part or parts of the body.

About stage group, stage 0 (T_{is} N_0 M_0); stage IA (T_1 N_0 M_0); stage IB(T_1 N_1 M_0), (T_2 N_0 M_0); stage IIA (T_1 N_2 M_0), (T_2 N_1 M_0), (T_3 N_0 M_0); stage IIB (T_1 N_{3a} M_0), (T_2 N_2 M_0), (T_3 N_1 M_0), (T_{4b} N_0 M_0); stage IIIA (T_2 N_{3a} M_0), (T_3 N_2 M_0), (T_{4a} N_1 M_0), (T_{4b} N_0 M_0); stage IIIB (T_1 or T_2 N_{3b} M_0), (T_3 N_{3a} M_0), (T_{4a} N_{3a} M_0), (T_{4b} N_1 or N_1 M_0); stage IIIC (T_3 or T_{4a} N_{3b} M_0), (T_{4b} N_{3a} or N_{3b} M_0); stage IV (any T or N, M_1)

Stage TNM and stage group of esophageal cancer[110]

T, N, and M status, histologic grade and stage group definitions for esophagus and esophagogastric junction cancer in the 7th edition of the American Joint Committee on Cancer Staging Manual

T status	
T_{is}	High-grade dysplasia
T1	Invasion into the lamina propria, muscularis mucosae, or submucosa
T2	Invasion into muscularis propria
T3	Invasion into adventitia
T4a	Invades resectable adjacent structures (pleura, pericardium, diaphragm)
T4b	Invades unresectable adjacent structures (aorta, vertebral body, trachea)
N status	
N0	No regional lymph node metastases
N1	1 to 2 positive regional lymph nodes
N2	3 to 6 positive regional lymph nodes
N3	7 or more positive regional lymph nodes
M status	
M0	No distant metastases
M1	Distant metastases
Histologic grade	
G1	Well differentiated
G2	Moderately differentiated
G3	Poorly differentiated
G4	Undifferentiated

Tab. 1.11: TNM stage of Gastric cancer

Stage	Adenocarcinoma				Squamous cell carcinoma				
	T	N	M	Grade	T	N	M	G	Location
0	is	0	0	1	is	0	0	1	Any
IA	1	0	0	1-2	1	0	0	1	Any
IB	1	0	0	3	1	0	0	2-3	Any
	2	0	0	1-2	2-3	0	0	1	Lower
IIA	2	0	0	3	2-3	0	0	1	Upper, middle
					2-3	0	0	2-3	Lower
IIB	3	0	0	Any	2-3	0	0	2-3	Upper, middle
	1-2	1	0	Any	1-2	1	0	Any	Any
IIIA	1-2	2	0	Any	1-2	2	0	Any	Any
	3	1	0	Any	3	1	0	Any	Any
	4a	0	0	Any	4a	0	0	Any	Any
IIIB	3	2	0	Any	3	2	0	Any	Any
IIIC	4a	1-2	0	Any	4a	1-2	0	Any	Any
	4b	Any	0	Any	4b	Any	0	Any	Any
	Any	3	0	Any	Any	3	0	Any	Any
IV	Any	Any	1	Any	Any	Any	1	Any	Any

Cancer location definitions: upper thoracic, 20-25 cm from incisors; middle thoracic, 25-30 cm from incisors; lower thoracic, 30-40 cm from incisors.

Tab. 1.12: Stage group of Gastric cancer

Depend the stage, tumor location and situation of patient, therapy options include local mucosal resection or ablation therapies, esophagectomy, chemotherapy, and radiation therapy[110]. For gastric cancer, also can use regimen of therapy by surgical, chemotherapy and radiation therapy[111].

The survival of *novo* esophageal cancer is not high, with a mortality (combined for esophageal and gastric cancer of 62.5%) being second only to that of lung cancer[96].

- Others novo neoplasia

Although it seems that breast cancer is no more frequent in LT compared to the general population, however, other studies have evidence that breast cancer incidence is in fact elevated in the transplant population, with the advantage, however, that early detection is more common, and this has also resulted in decreased mortality compared to that of the general population upon similar diagnose. The stage of breast cancer is complicated, including stage TNM and stage group[96,112].

TX: The primary tumor cannot be evaluated.

T0: There is no evidence of cancer in the breast.

Tis: Refers to carcinoma in situ. The cancer is confined within the ducts or lobules of the breast tissue and has not spread into the surrounding tissue of the breast. There are 2 types of breast carcinoma in situ:

Tis (DCIS): DCIS is a noninvasive cancer, but if not removed it may develop into an invasive breast cancer later. DCIS means that cancer cells have been found in breast ducts and have not spread past the layer of tissue where they began.

Tis (Paget's): Paget's disease of the nipple is a rare form of early, noninvasive cancer that is only in the skin cells of the nipple. Sometimes Paget's disease is associated with another, invasive breast cancer. If there is another invasive breast cancer, it is classified according to the stage of the invasive tumor.

T1: The tumor in the breast is 20 mm or smaller in size at its widest area. This is a little less than an inch. This stage is then broken into 4 substages depending on the size of the tumor:

T1mi is a tumor that is 1 mm or smaller

T1a is a tumor that is larger than 1 mm but 5 mm or smaller

T1b is a tumor that is larger than 5 mm but 10 mm or smaller

T1c is a tumor that is larger than 10 mm but 20 mm or smaller

T2: The tumor is larger than 20 mm but not larger than 50 mm.

T3: The tumor is larger than 50 mm.

T4: The tumor falls into 1 of the following groups:

T4a means the tumor has grown into the chest wall.

T4b is when the tumor has grown into the skin.

T4c is cancer that has grown into the chest wall and the skin.

T4d is inflammatory breast cancer.

NX: The lymph nodes were not evaluated.

N0: Either of the following: No cancer was found in the lymph nodes. Only areas of cancer smaller than 0.2 mm are in the lymph nodes.

N1: The cancer has spread to 1 to 3 axillary lymph nodes and/or the internal mammary lymph nodes.

N2: The cancer has spread to 4 to 9 axillary lymph nodes. Or it has spread to the internal mammary lymph nodes, but not the axillary lymph nodes.

N3: The cancer has spread to 10 or more axillary lymph nodes. Or it has spread to the lymph nodes located under the clavicle, or collarbone. It may have also spread to the internal mammary lymph nodes. Cancer that has spread to the lymph nodes above the clavicle, called the supraclavicular lymph nodes, is also described as N3.

MX: Distant spread cannot be evaluated.

M0: The disease has not metastasized.

M0 (i+): There is no clinical or radiographic evidence of distant metastases. Microscopic evidence of tumor cells is found in the blood, bone marrow, or other lymph nodes that are no larger than 0.2 mm.

M1: There is evidence of metastasis to another part of the body, meaning there are breast cancer cells growing in other organs.

Stage 0	T_{is}	N_0	M_0
Stage IA	T_1	N_0	M_0
Stage IB	T_0 or T_1	N_1	M_0
Stage IIA	T_0	N_1	M_0
	T_1	N_1	M_0
	T_2	N_0	M_0
Stage IIB	T_2	N_1	M_0
	T_3	N_0	M_0
Stage IIIA	T_0-T_3	N_2	M_0
	T_3	N_1	M_0
Stage IIIB	T_4	N_0-N_2	M_0
Stage IIIC	Any T	N_3	M_0
Stage IV	Any T	Any N	M_1

Tab. 1.13: Stage group of Breast cancer

Due to the mentioned above, the stage TNM and stage group of kidney cancer and prostate cancer are not more detailed description. The incidence of prostate cancer does not seem to be increased in LT recipients, but the other genitourinary cancers (especially the renal cancer) seem to be higher than that of the general population. Mean time to diagnosis of non-prostate genitourinary cancer ranges from 20 to 55.3 months, while in cases of prostate cancer the diagnosis is often performed between 5.8 and 18.4 months after LT. In LT recipients, prostate cancer is more often diagnosed at earlier stages and has a good prognosis, whereas renal and bladder cancers have a poor prognosis[96].

2. HYPOTHESIS

2. Hypothesis

- The use of mTORi in patients with liver transplantation caused by HCC reduces the incidence of recurrence and increases the survival rate.

- The use of mTORi in the patient after liver transplantation with *de novo* neoplasia reduces the incidence of recurrence and increasing the survival rate.

3. OBJECTIVE

3. Objective

1. To analyze if the use of mTORi in the patient with liver transplantation caused by HCC reduces the incidence of recurrence of HCC and increase the survival rate.

2. To analyze if the use of mTORi in the patient with liver transplantation and "*de novo"* neoplasia reduces the incidence of recurrence and increase the survival rate.

4. METHODS

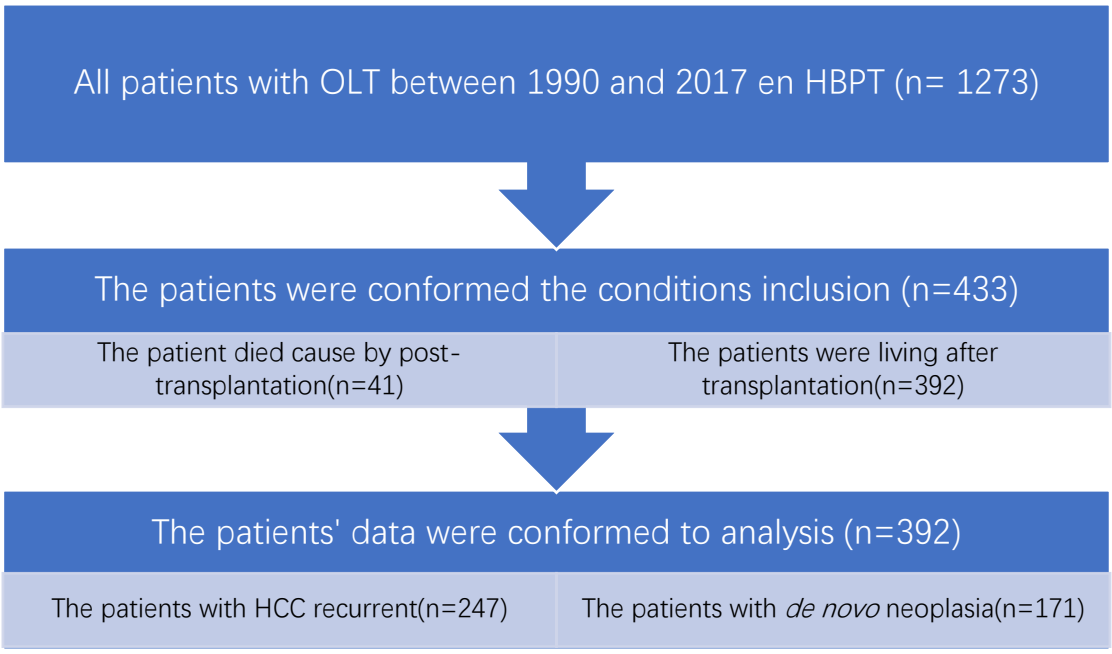
4. Methods

Clinical study with liver transplant recipients operated between 1990 and 2017 because of HCC or liver transplants recipients with “de novo” neoplasia. The methodology of the work has followed the order of the hypotheses and objectives of the same.

- To analyze if the use of mTORi in the patient with liver transplantation caused by HCC reduces the incidence of recurrence of HCC and increase the survival rate.

- To analyze if the use of mTORi in the patient with liver transplantation and “*de novo”* neoplasia reduces the incidence of recurrence and increase the survival rate.

This was an observational, retrospective cohort study, using a sample of patients drawn from all 1273 liver transplant patients at the Virgen del Rocío Hospital between March 1990 and July 2017. Of these, 433 patients were selected.



Tab. 4.1. The patients selection

The table 1. describes how to conformed the patients and the types of patients for analysis. The patients conformed the conditions inclusion were 433. More than 90% of these patients were selected. 41 patients died post-transplantation, so were excluded. In these 392 patients, 247 patients were diagnosis with HCC and recurrent HCC. And there are 171 patients with de novo neoplasia.

About patients

In the tables below is shown the distribution of patients

Gender of patients		
Gender	**Frequency**	**Percentage**
Male	371	85.70%
Female	62	14.30%

The male patients were 6 times of female patients, it may caused because of alcohol intaking and smoke usually happens in the population male.

Gender of patients whit HCC and *de novo* neoplasia(NN)				
Gender	Frequency(HCC)	Percentage(HCC)	Frequency(NN)	Percentage(NN)
Male	211	85.40%	148	86.55%
Female	36	14.60%	23	13.45%
Total	247	100%	171	100%

Tab. 4.2. and Table 4.3. The distribution of gender of patients

Without the patients exclusion, the proportion of male/female in the patients with HCC and the patients *de novo* neoplasia are almost same that the male patients are 7 times of female. And the p > 0,05.

Cirrhosis etiology		
Type	Frequency	Percentage
Alcoholic Cirrhosis	245	56.60%
CHVC	93	21.46%
CHVB	57	13.16%
Others	38	8.78%
Total	433	100%

Cirrhosis etiology of patients with HCC and the patients with de novo neoplasia(NN)					
Type	Frequency(HCC)	Percentage(HCC)	Frequency(NN)	Percentage(NN)	*p*
Alcoholic Cirrhosis	134	54.25%	112	65.50%	>0,05
CHVC	66	26.72%	18	10.52%	>0,05
CHVB	33	13.36%	19	11.11%	>0,05
Others	14	5.67%	22	12.87%	>0,05

Tab. 4.4. and Table 4.5. The distribution of diagnosis

Most of HCC were caused by alcoholic cirrhosis, also hepatitis virus B and C can affect HCC too. Obviously, there are some other cause in the table. 4, but there are rare cases which were less than 10% of it.

Distribution of age of the patients		
Age	Frequency	Percentage
20-50	75	19.40%
51-60	197	49.90%
61-70	120	30.70%

Tab. 4.6. The distribution of age of patients at the moment of liver transplant

Most patients who make the operation of transplantation were between 51-60 years, and the mean was 56.04 years old.

Blood group		
Type	Frequency	Percentage
O	162	37.40%
A	187	43.20%
B	60	13.80%
AB	24	5.60%

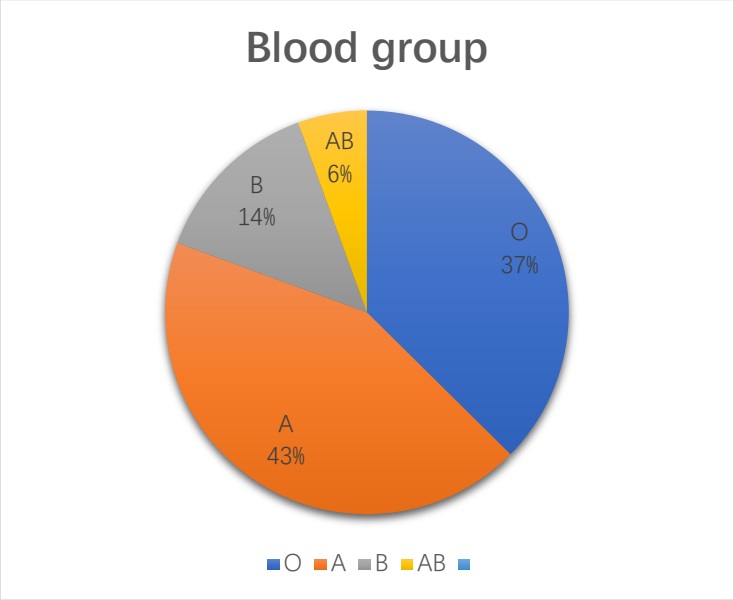


Fig. 4.1

Tab. 4.7. and Fig 4.1. The distribution of Blood Group

Blood group of the patients with HCC and patients with de novo neoplasia(NN)					
Type	Frequency(HCC)	Percentage(HCC)	Frequency(NN)	Percentage(NN)	*p*
O	97	39.27%	61	35.68%	>0,05
A	106	42.91%	71	41.52%	>0,05
B	34	13.77%	28	16.37%	>0,05
AB	10	4.05%	11	6.43%	>0,05

Tab. 4.7-8. and Fig. 4.1. The distribution of Blood group

Smoke		
Type of smoke	Frequency	Percentage
Yes	287	66.30%
No	146	33.70%

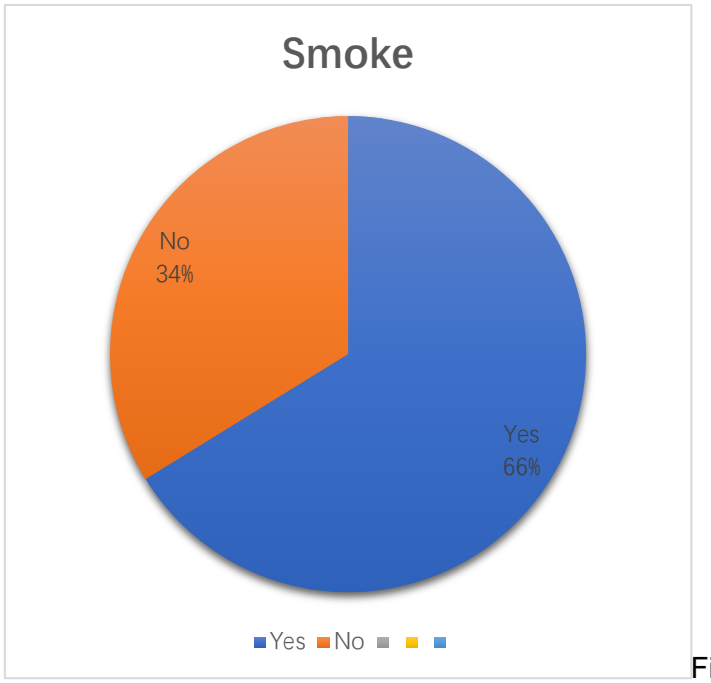


Fig. 4.2

Tab. 4.9 and Fig 4.2. Distribution of smoking in patients of study

Distribution of smoke in patients with HCC and NN				
Type	Frequency(HCC)	Percentage(HCC)	Frequency(NN)	Percentage(NN)
Yes	170	68.80%	121	70.80%
No	77	31.20%	50	29.20%

Tab. 4.9-10. and Fig. 4.2. The distribution of smoke in patients

In general, the patients with smoke is 2 times higher than without it. There are no difference between HCC and de novo neoplasia patient.

Alcohol		
Type	Frequency	Percentage
Yes	278	64.20%
No	154	35.60%

Distribution of alcohol in patients with HCC and NN					
Type	Frequency(HCC)	Percentage(HCC)	Frequency(NN)	Percentage(NN)	*p*
Yes	168	68%	116	67.80%	>0,05
No	79	32%	55	32.20%	>0,05

Tab. 4.11-12. The distribution of alcohol in patients

As mentioned above, the patient who smoking always intaking alcohol. There are no difference between HCC and de novo neoplasia.

Organ appearance		
Type	Frequency	Percentage
Optimum	303	70%
Suboptimal	130	30%

Tab. 4.13. The organ appearance of donor

En these patients, there are 247 patients with HCC recurrent, and 171 patients with *de novo* neoplasia after liver transplantation. Including colorectal cancer, lung cancer, kidney cancer et.al.

The distribution of *de novo* neoplasia		
Type of cancer	Frequency	Percentage
Lung cancer	28	16.37%
Colorectal cancer	8	4.68%
Urothelial cancer	4	2.34%
Esophagus cancer	1	0.58%
Pancreatic cancer	2	1.17%
Small bowel cancer	2	1.17%
Hepatic sarcoma	1	0.58%
Otorhinolaryngologic cancer	31	18.13%
Lymphoproliferative syndrome	14	8.19%
Breast cancer	6	3.51%
Prostate cancer	10	5.85%
Kidney cancer	4	2.34%
Skin cancer	55	32.16%
Gastric cancer	3	1.75%
Central nervous cancer	1	0.58%
Cholangiocarcinoma	1	0.58%

Tab. 4.14. The types of cancer in *de novo* neoplasia

In the patients with *de novo* neoplasia, there are 69 patients with tumor recurrence.

Immunosuppressive agent	Frequency	Percentage
mTORi	152	35.1%
CNI	255	58.9%
MMF	26	6%

Tab. 4.15. Distribution of immunosuppressive agent

Immunosuppressive agent	Frequency	Percentage
mTORi	86	34.8%
CNI	158	1.2%
MMF	3	64%

Tab. 4.16. Immunosuppressive agent use in HCC

Immunosuppressive agent	Frequency	Percentage
mTORi	77	45%
CNI	69	14.6%
MMF	25	40.4%

Tab. 4.17. Immunosuppressive agent use in “de novo” neoplasia

Clinical study variables

The criteria of safety assessment, and efficacy were determined after the follow-up many years. We record the side effects of mTORi.

The efficacy variables of the preservation of *de novo* neoplasia:

1. Survival
2. Recurrence rate.

Statistical analysis of clinical study

For the purposes of the present analysis, the data were extracted from the database in the form of cohorts for both CNI and mTORi (including Sirolimus and Everolimus). From the clinical and demographic point of view, the data collected were: age, sex, etiology of liver disease, presence of hepatocellular carcinoma, alcohol, smoke, date of transplant, cancer stage, date of diagnosis *de novo* neoplasia, Child-Pugh, MELD Score. And a complete history of immunosuppressive medication including terms and duration of treatment with mTORi was also obtained.

The analyzes were performed with the IBM SPSS Statistics version 23.0 program. we have performed descriptive statistics of the study variables. For this we have used absolute frequencies and percentage in the case of qualitative variables. The quantitative variables will be summarized by Md (SD) (mean, standard deviation) and range (minimum and maximum) or P50 [P25 - P75] (median, interquartile range), depending on the degree of asymmetry of the same. The comparison of the qualitative variables was carried out by means of analysis with Chi^2 text. And the survival analysis has been carried out with the nonparametric Kaplan-Meier method.

A value of $P < 0.05$ was considered statistically significant, using the two-tailed test.

5. RESULTS

5. Results

5.1. Hepatocellular carcinoma(HCC)

5.1.1. Influence of smoking and alcohol consumption

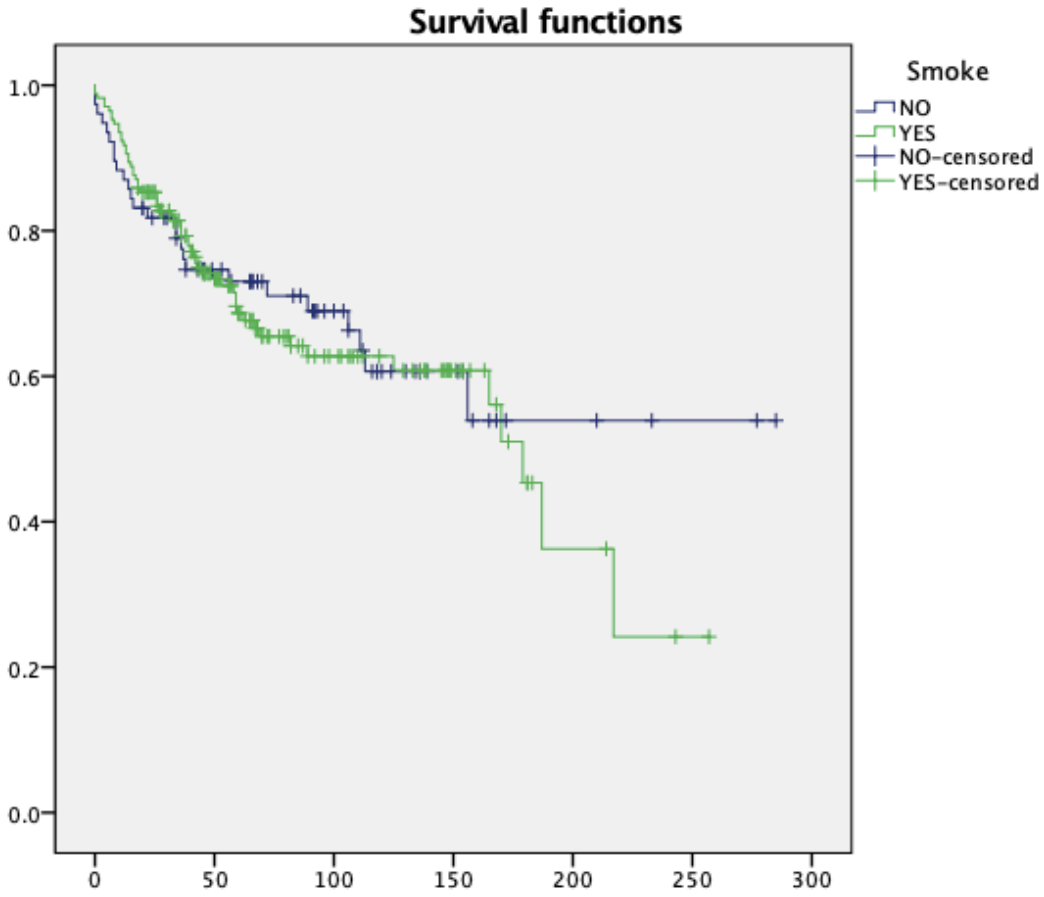


Figure 5.1. The survival rate of smoke in patients with HCC (unit: month)

We use the test Kaplan-Meier (p=0.670, p>0.05), in the short-term, the influence of smoke in survival is not clear. But in the long-term, the survival rate of patient without smoke is better.

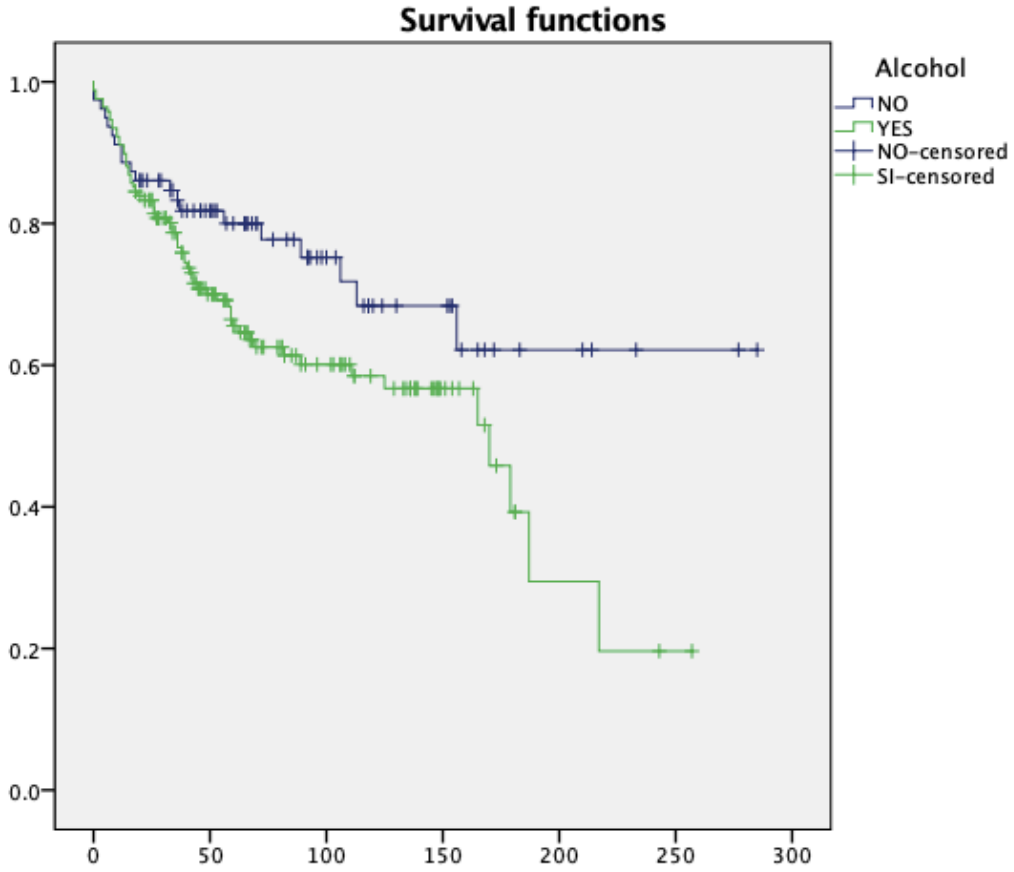


Figure 5.2. The survival rate of alcohol in patients with HCC (unit: month)

Also make the test to confirm the survival rate of alcohol intaking. And with the p=0.035 (p<0.05). For intaking alcohol, whatever short-term or long-term, no intake alcohol is much better than alcoholic intake. More than 60% patients without alcohol intaking and theirs' survival time can achieve to 300 months, lees than 20% patients with alcohol intaking can reach to 200 months.

5.1.2. Histological Findings

There 247 patients with the diagnosis HCC, and the study has mentioned the distribution of gender, type of cirrhosis, blood group, smoke and alcohol of patients in the part method. But the study also describe the others side of HCC. Like the vascular invasion, capsule appearance and the differentiated of HCC.

Vascular invasion

		Frequency	Percentage	Percentage valid	Percentage accumulated
Valid	NO	206	83.4	83.4	83.4
	SI	41	16.6	16.6	100.0
	Total	247	100.0	100.0	

Table 5.1. The distribution of Vascular invasion in patients with HCC

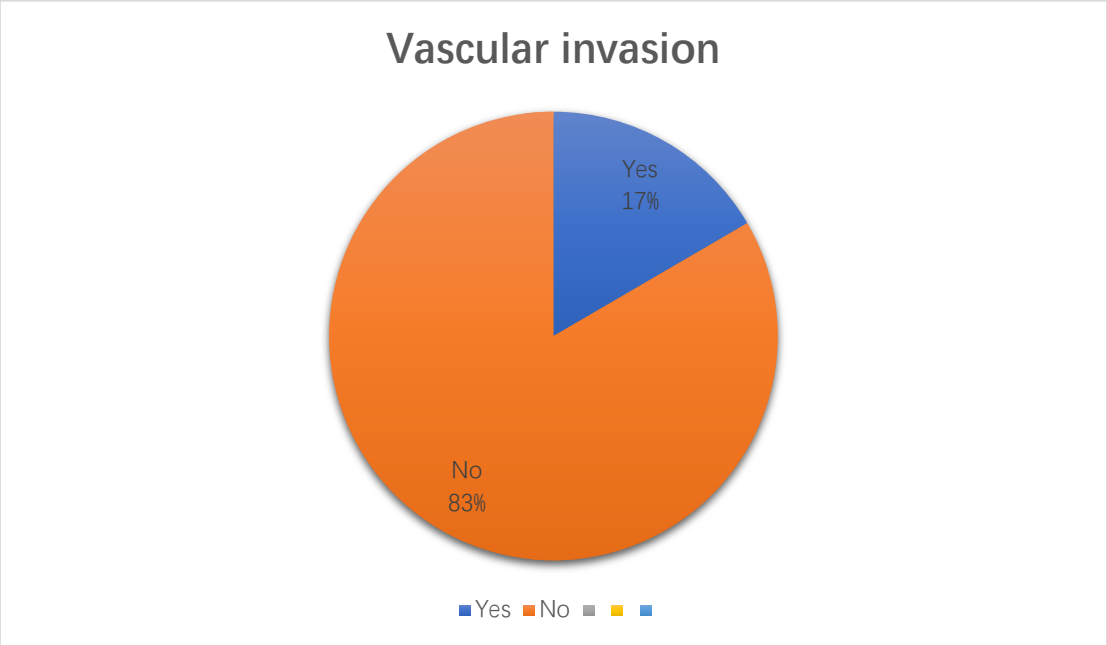


Figure 5.3. vascular invasion

The Table 5.1. and figure 5.3. shown the patients with HCC and the distribution of Vascular invasion.

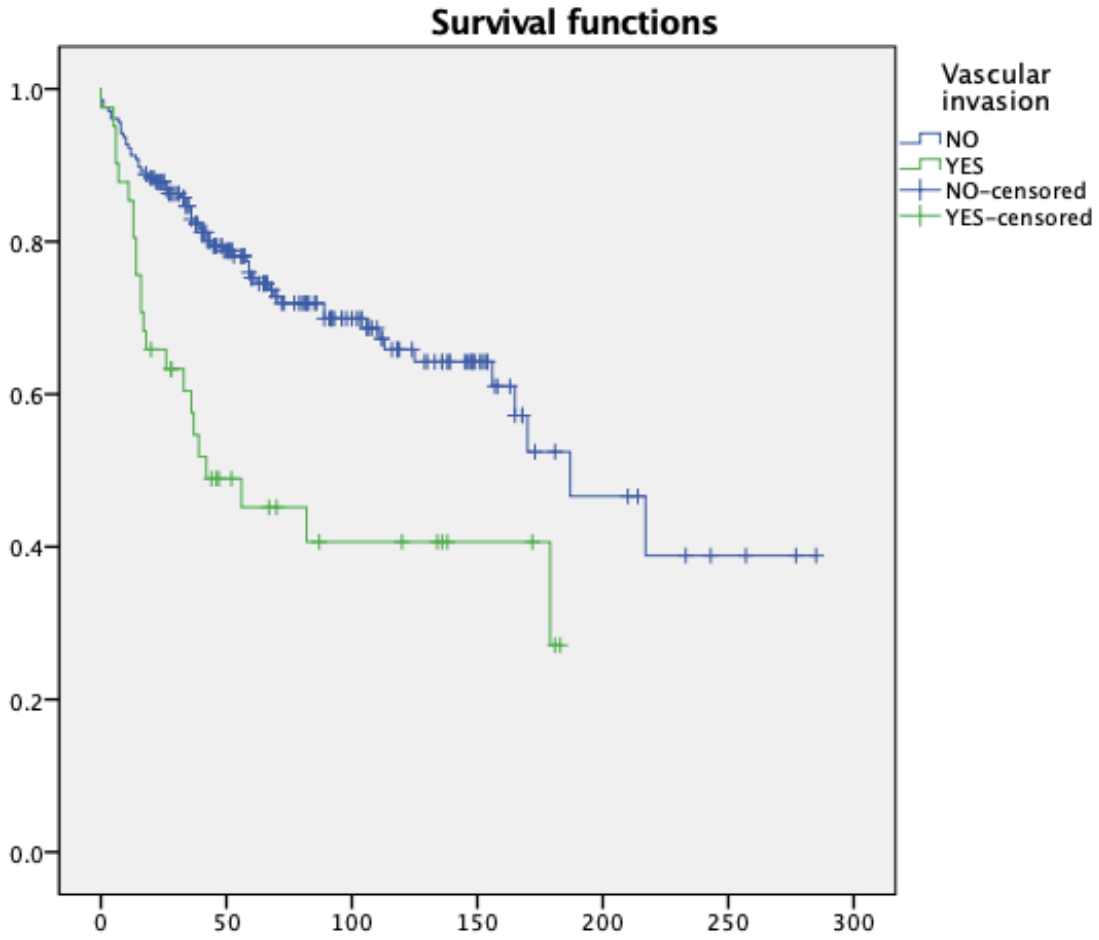


Figure 5.4. The survival rate of Vascular invasion in patients with HCC (unit: month)

With the test ($p<0.0001$, $p<0.05$) Kaplan-Meier, known about the related between survival time and the vascular invasion, almost 80% patients without vascular invasion reach 50 months, and more than 60% patients reach 150 months. However, just 40% patients can reach 220 months. Other side, less than 50% patients with vascular invasion can reach 50 months, and the largest time of survival is 183 months, just 3 (7.3%)patients' survival are more than 150 months.

Capsule appearance

		Frequency	Percentage	Percentage valid	Percentage accumulated
Valid	NO	215	87.0	87.0	87.0
	SI	32	13.0	13.0	100.0
	Total	247	100.0	100.0	

Table 5.2. The distribution of capsule appearance in patients with HCC

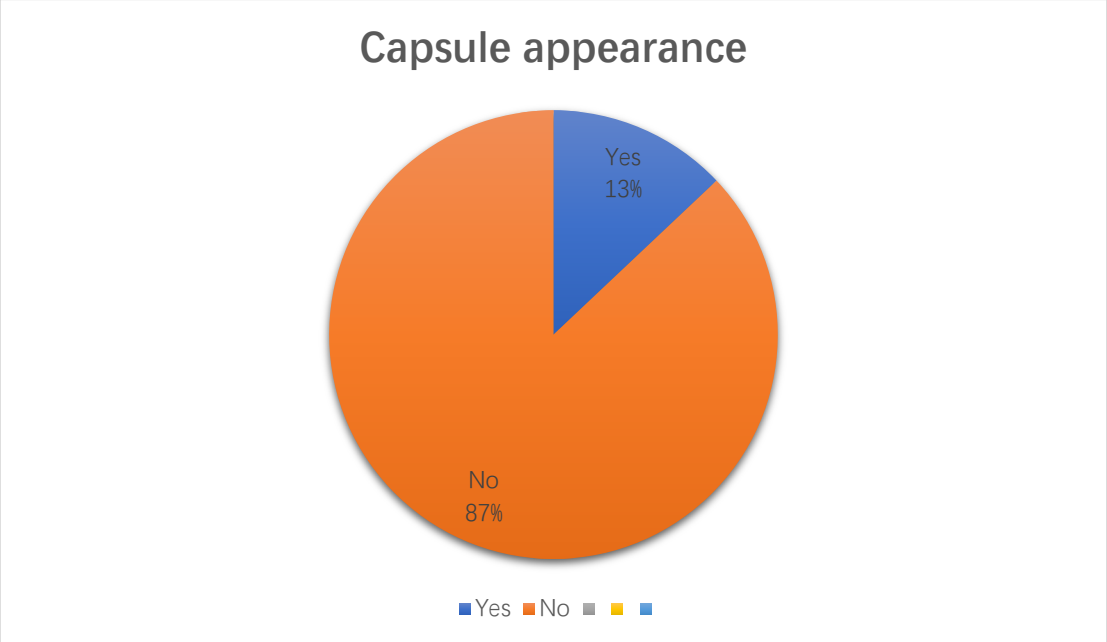


Figure 5.5. capsule appearance of HCC

With the table 2. and the figure 5. Just 32 patients with capsule appearance. In the figure 6. (Test Kaplan-Meier, with p<0.0001, <0.05)shown the patients without capsule appearance, the survival time is much better than the patients with capsule

appearance. More than 60% patients without it achieve 150 months after liver transplantation, however, the patients with it just 1 patient's (3.12%) survival time more than 150 months. Most of these patients with it, the survival time is about 50 months

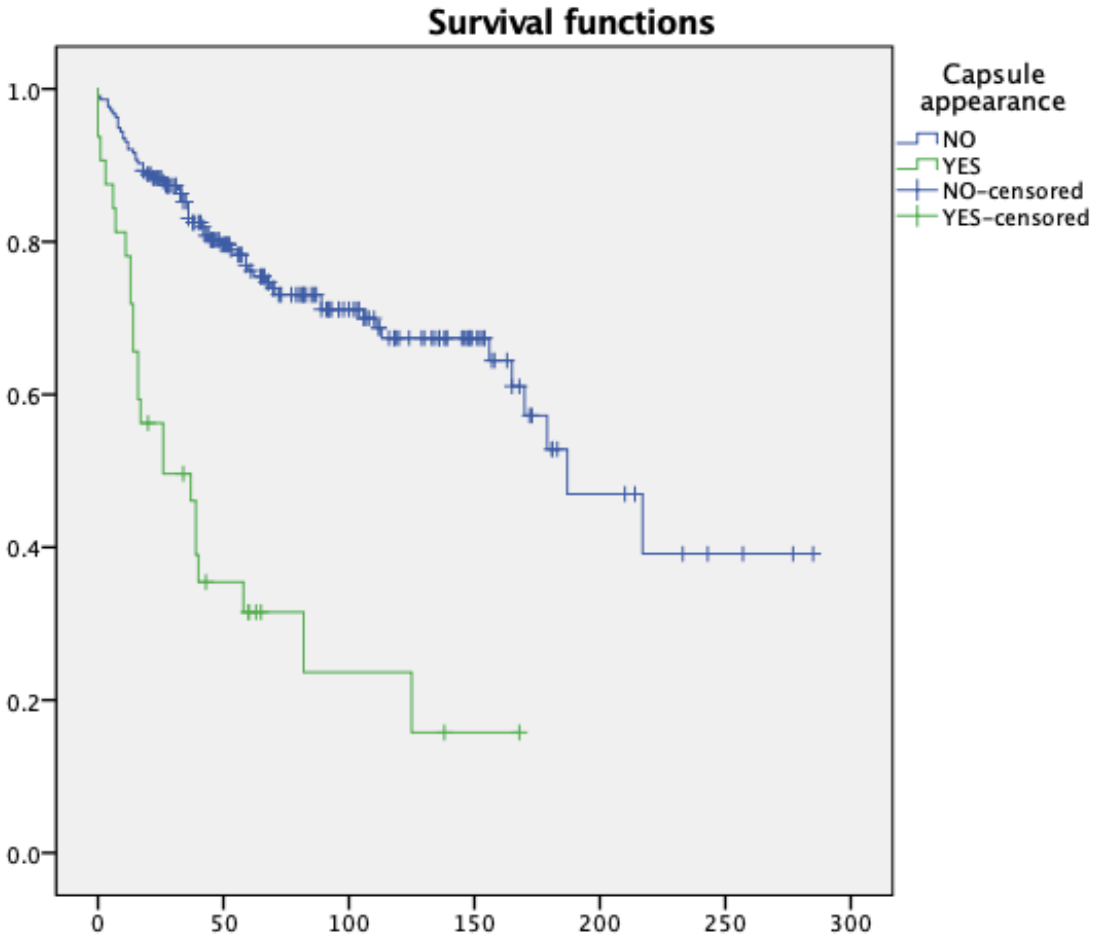


Figure 5.6. The survival rate of Capsule appearance in patients with HCC (unit: month)

Edmondson

		Frequency	Percentage	Percentage valid	Percentage accumulated
Valid	High differentiated	36	14.6	14.6	14.6
	Moderately differentiated	189	76.5	76.5	91.1
	Poorly differentiated	22	8.9	8.9	100.0
	Total	247	100.0	100.0	

Table 5.3. The differentiated of HCC

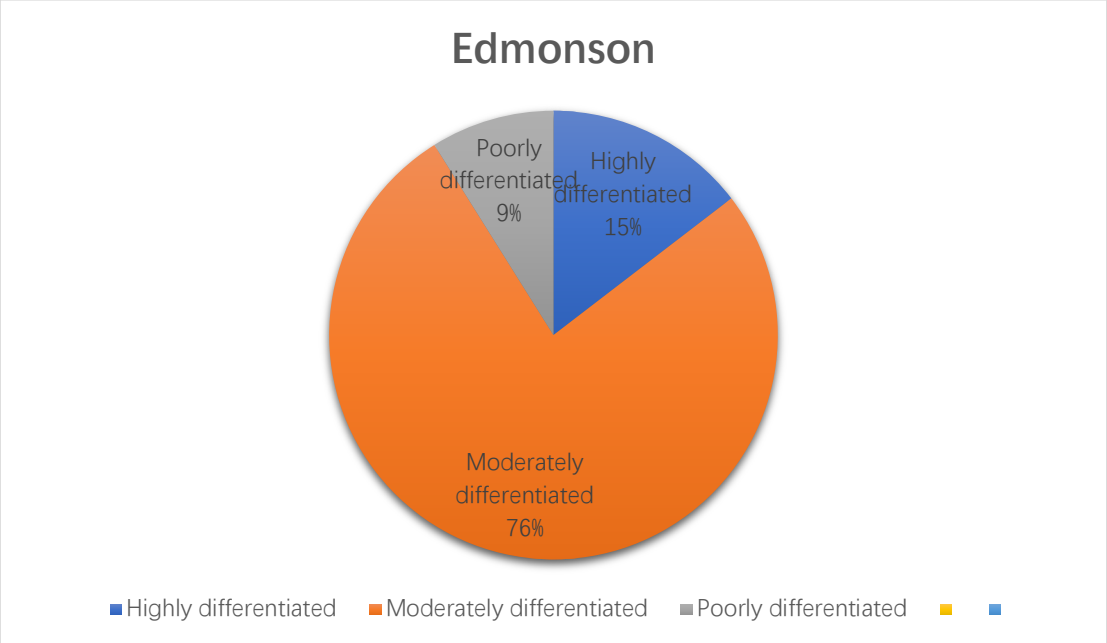


Figure 5.7. The percentage of Edmondson of HCC

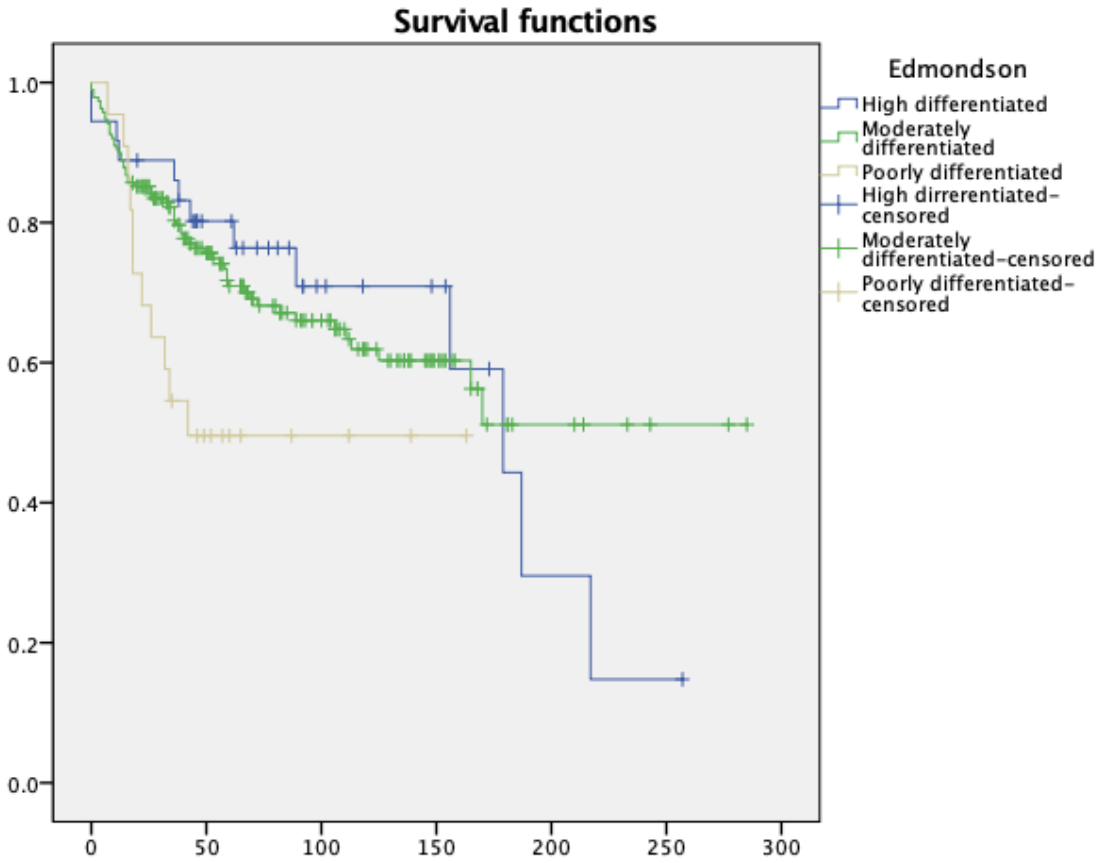


Figure 5.8. The survival rate of Edmondson in patients with HCC(unit: month)

With the table. 3 and figure. 7, can shown directly the distribution of the differentiated of cancer in patients. The figure 8. is the test Kaplan-Meier with p=0.090 (p>0.05). In general, the tumor with highly differentiated is much better than poorly differentiated. The moderately differentiated is between those.

5.1.3. Tumor recurrence

247 patients with the diagnosis HCC, and there are 42 patients occur HCC recurrent, 26 of it were metastasis.

Tumor recurrence

		Frequency	Percentage	Percentage valid	Percentage accumulated
Valid	NO	205	83.0	83.0	83.0
	SI	42	17.0	17.0	100.0
	Total	247	100.0	100.0	

Table 5.4. HCC recurrence in patients

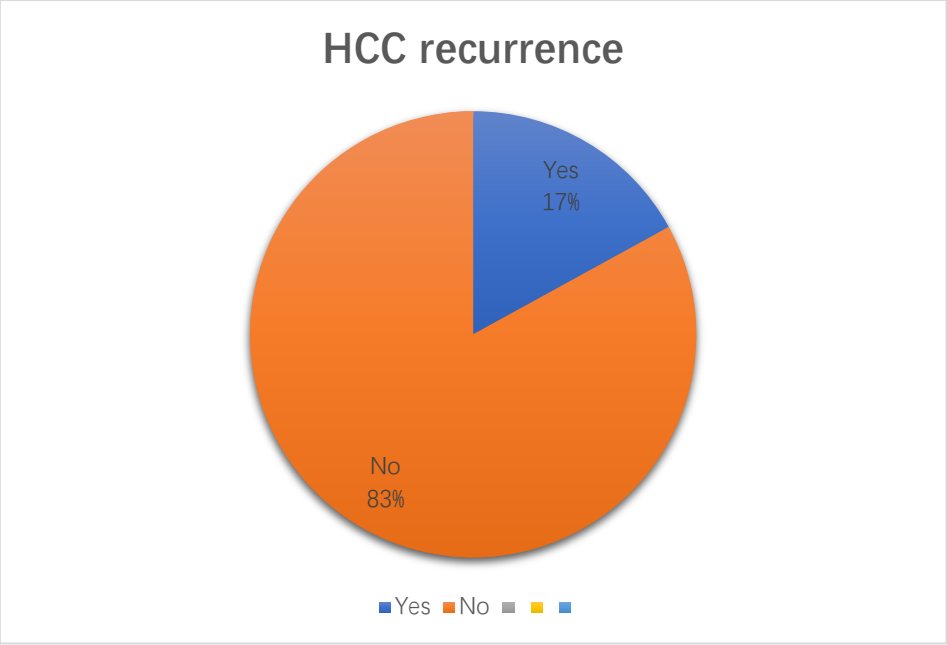


Figure 5.9. The percentage of HCC

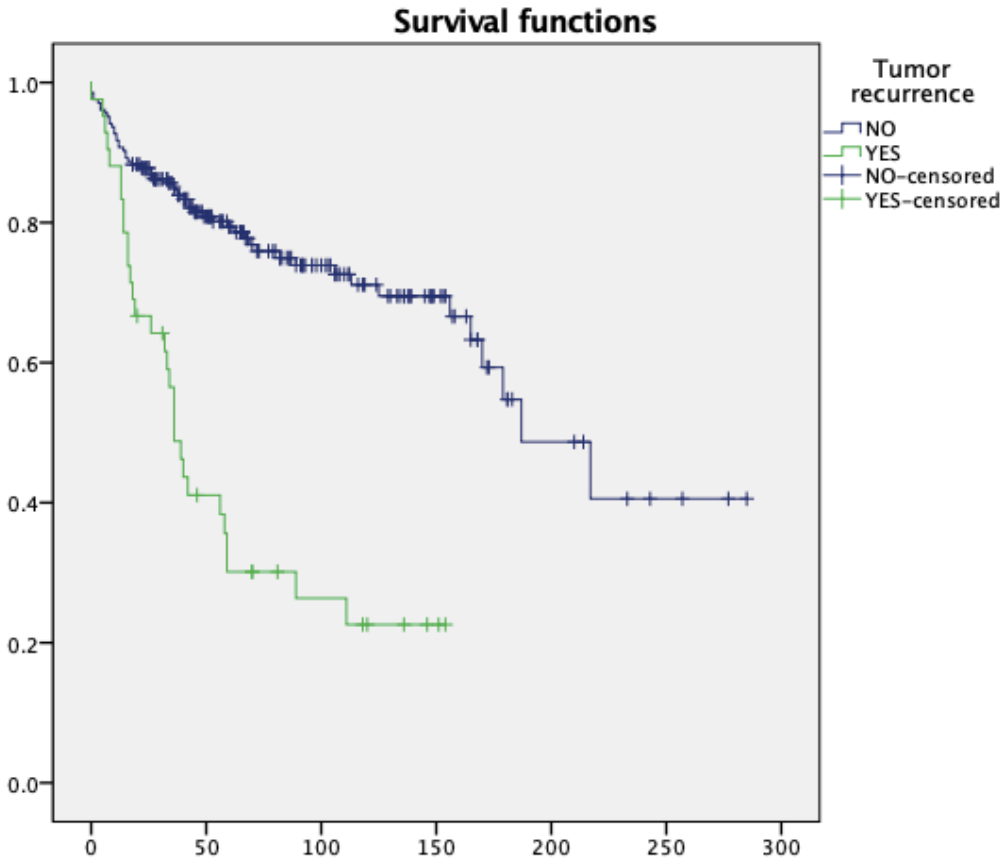


Figure 5.10. Survival rate of patients with and without recurrence.(unit: month)

We use test Kaplan-Meier to confirm the survival rate of recurrence tumor ($p<0.0001$, $p<0.05$). The survival rate of patients without HCC recurrent is higher than the patients with HCC recurrent. And 40% of these can achieve 300 months.

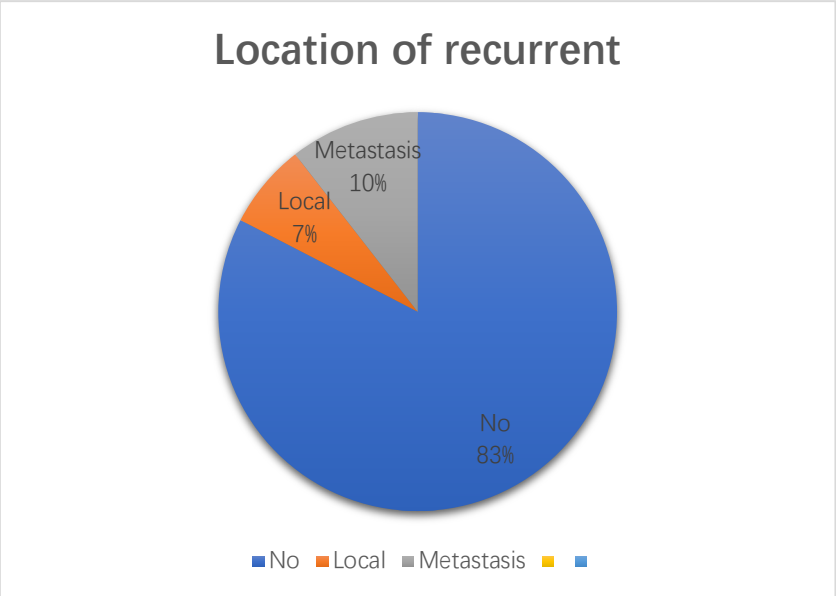


Figure 5.11. The distribution of location of recurrent

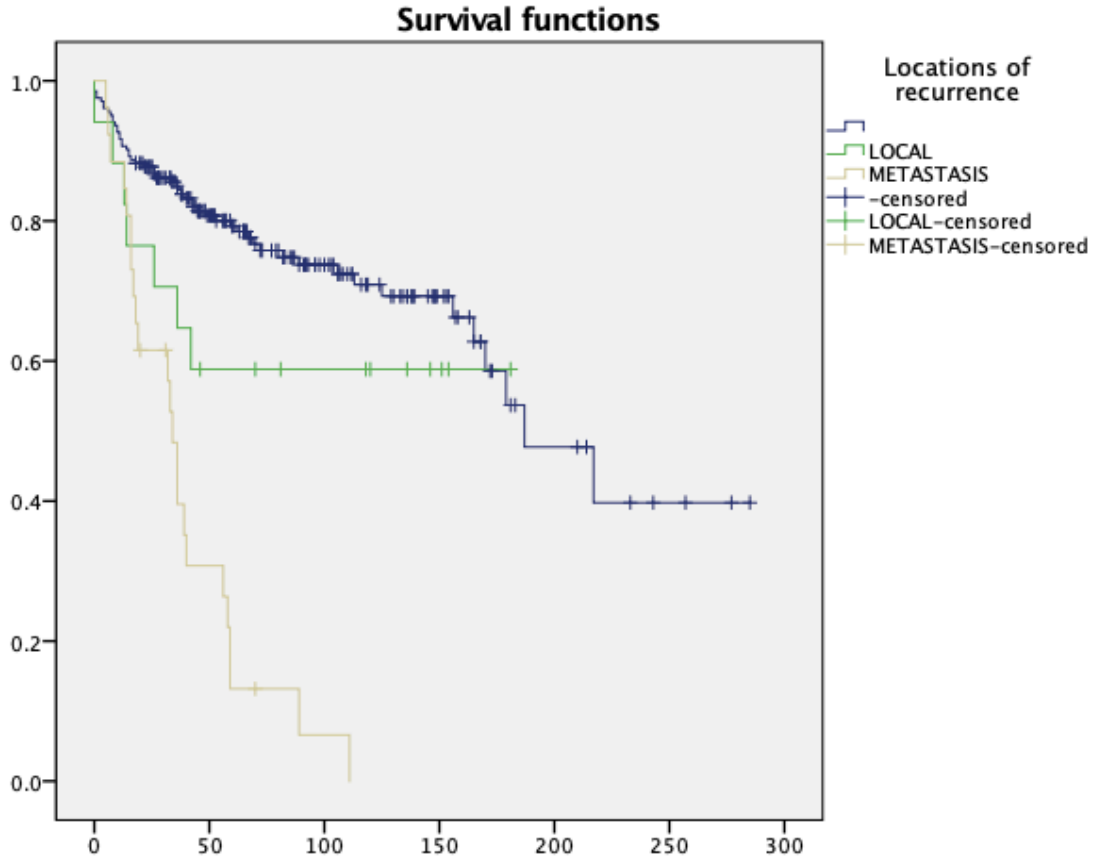


Figure 5.12. The survival rate of the location of recurrent tumor (unit: month)

Due to the figure 11. Most recurrence are metastasis. But in the figure 12 (test with $p<0.0001$, $p<0.05$), the patients without recurrence and the survival time are much better than other two. About 70% patients reach 150 months (12.5 years), more than 80% patients achieve 50 months, 40% patients' survival time more than 200 months. 60% patients with recurrent tumor in location local can reach 50 months, but the patients with metastasis just 30% of these can reach 50 months.

5.1.4. Immunosuppressant scheme

There are 158 patients use CNI, 86 patients with mTORi (37 patients use Sirolimus and 49 patients use Everolimus) and 3 patients with mycophenolate.

mTORi, CNI, MMF

		Frequency	Percentage	Percentage valid	Percentage accumulated
Valid	mTORI	86	34.8	34.8	34.8
	CNI	158	64.0	64.0	98.8
	MMF	3	1.2	1.2	100.0
	Total	247	100.0	100.0	

Table 5.5. The use of immunosuppressive inhibitor

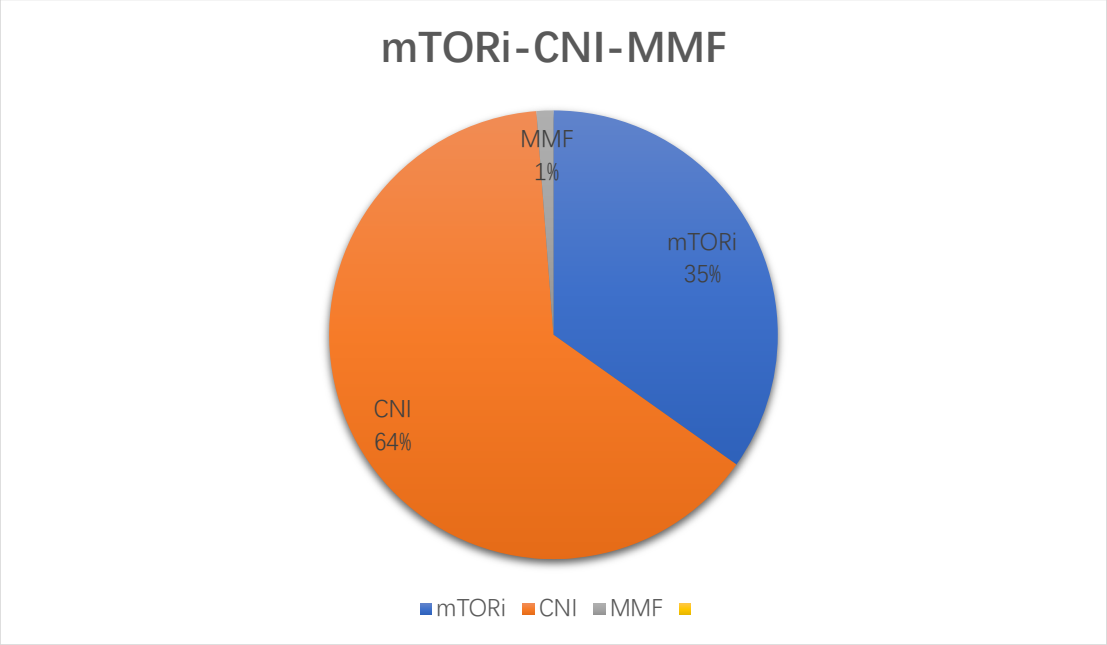


Figure 5.13. The survival of patients transplanted because of HCC in function of immunosuppressant regimen

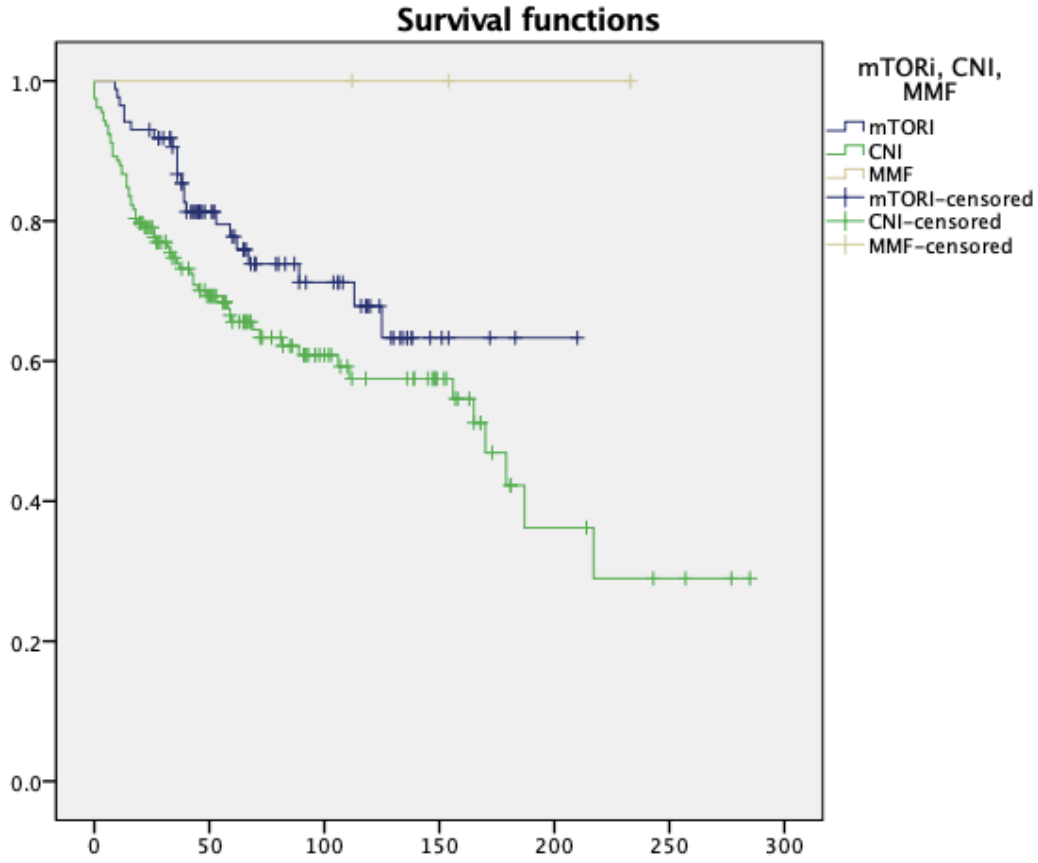


Figure 5.14. The survival rate of patients with CNI, mTORi and MMF. (unit: month)

The figure 14. is describe the survival rate by test Kaplan-Meier with the p=0.051. To the patients after liver transplantation, the effective of mTORi is better than CNI. More than 80% patients' survival time beyond 50 months who used mTORi. The patients with CNI, about 70%, even less than 70% achieve 50 months. 60% patients with mTORi achieve 200 months, less than 40% patients with CNI reach 200 months and rare patients' survival time reach 250 months.

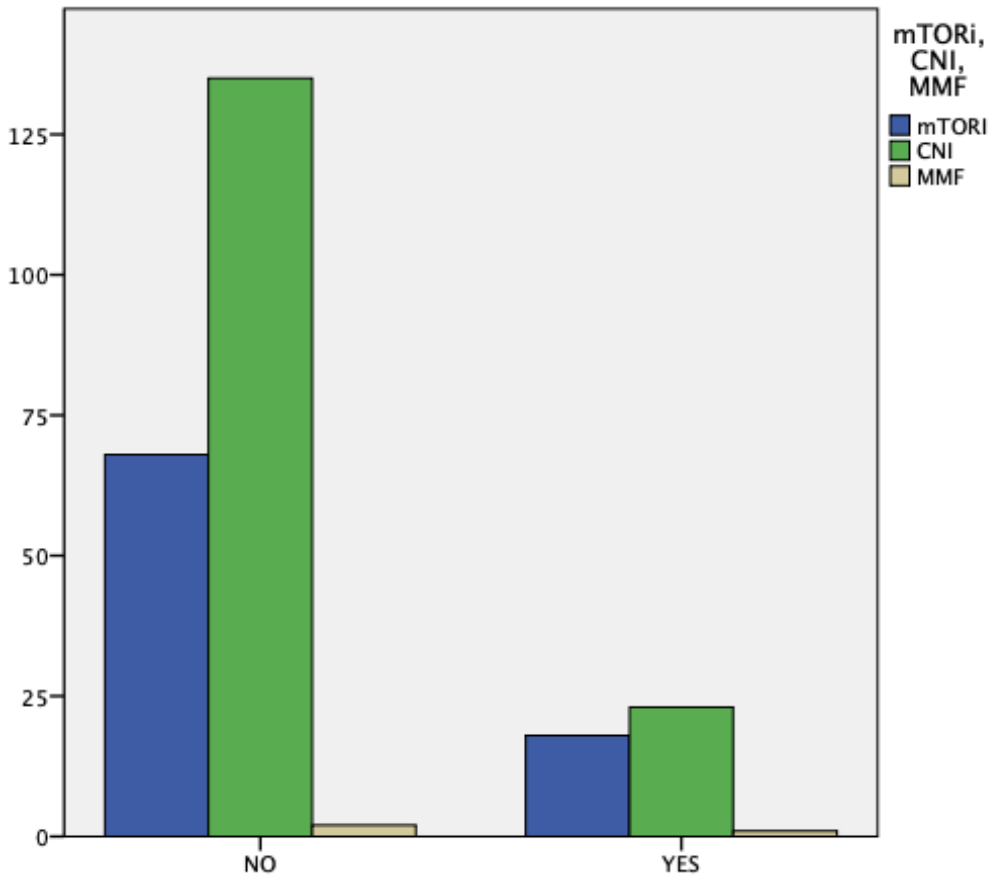


Figure 5.15. The distribution of patient used immunosuppressant with or without HCC recurrence.

In the figure 5.15. we can see 42 patients with HCC recurrence (18 patients with mTORi, 23 patients with CNI and 1 patient with MMF). (p=0.337)

SRL and EVR

		Frequency	Percentage	Percentage valid	Percentage accumulated
Valid		161	65.2	65.2	65.2
	SRL	37	15.0	15.0	80.2
	EVR	49	19.8	19.8	100.0
	Total	247	100.0	100.0	

Table 5.6. Compare the Sirolimus and Everolimus in patients use mTORi

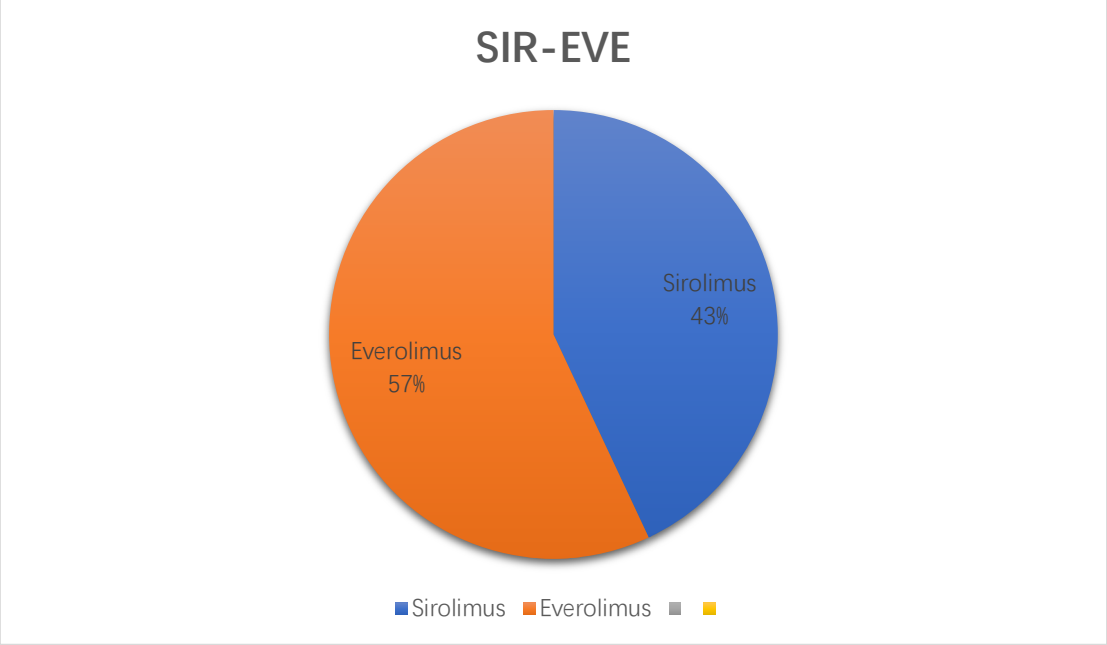


Figure 5.16. The survival of patients about distribution of mTORi (Sirolimus and Everolimus)

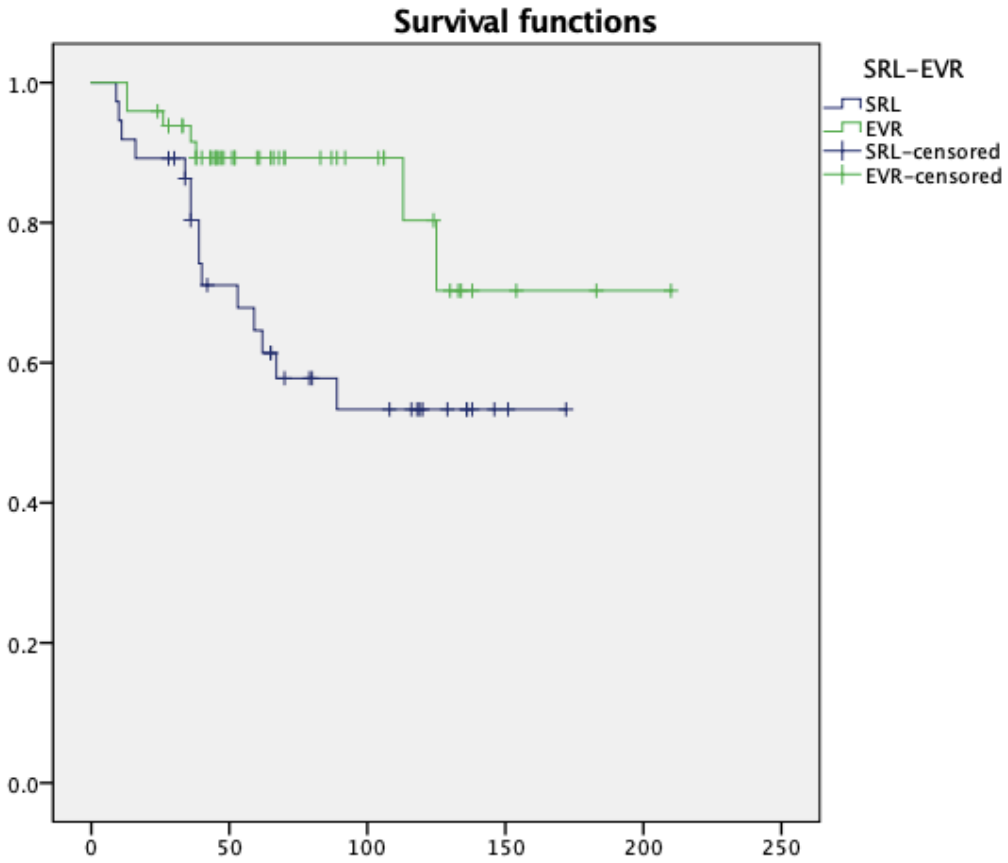


Figure 5.17. The survival rate of patients use Sirolimus and Everolimus (unit: month)

In the 86 patients use mTORi, there are 37 patients use Sirolimus and 49 patients use Everolimus. Figure 16. compare these two inhibitor's effect and the survival time. The test with the $p=0.018$ ($p<0.05$). Due to the test and the figure, the effect of Everolimus is better than Sirolimus. The most difference is in the point 100 months, about 40%, but continue to 125 months, the difference is about 20%.

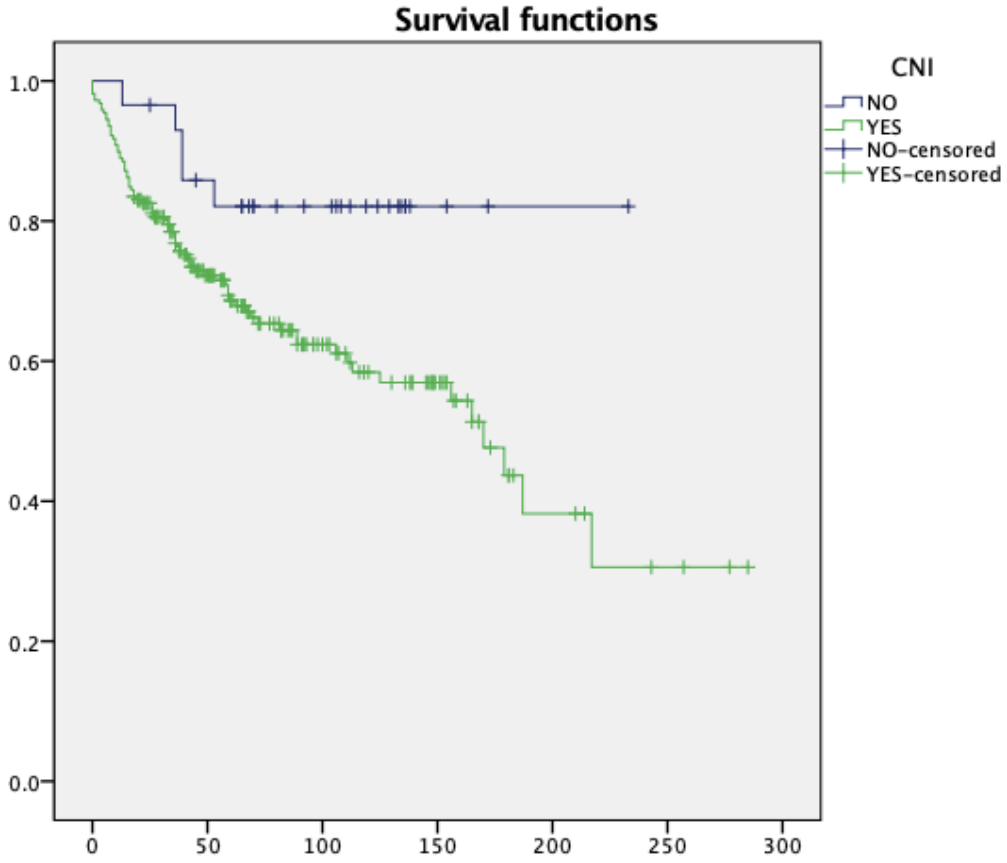


Figure 5.18. The survival rate of the patients with and without CNI. (unit: month)

Also making the survival test to the patients with and without CNI's therapy. This test with the p=0.025 (p<0.05). The test shown directly that the patients without CNI's therapy is better than with CNI's therapy, although there are rare patients' survival time larger than 250 months with CNI.

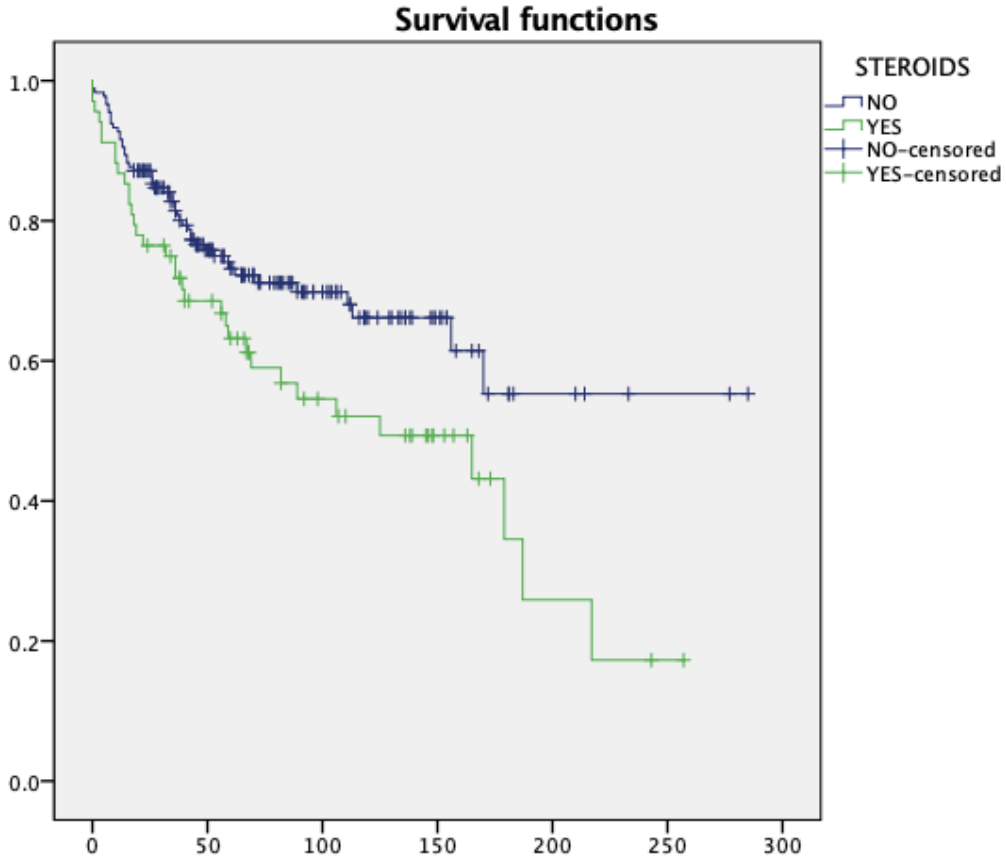


Figure 5.19. The survival rate of the patients with and without steroid therapy. (unit: month)

Steroid therapy also is a way to control the rejection reactive. We use the survival test (Kaplan-Meier, with the p=0.018 (p<0.05)) to compare the result of with and without steroid therapy. Same to the CNI's therapy, the patients without steroid therapy is better than with steroid therapy.

We also make the multivariate test to confirm the results, in the Tab. 5.7. we can see the valor of these variables.

Multivariate test

Effect	Valor
mTORi, CNI, MMF	*p<0.0001*
SRL, EVR	*p=0.411*
Alcohol	*p=0.116*
Smoke	*p=0.068*
Stage T	*p<0.0001*
Stage N	*p<0.0001*
Stage M	*p<0.0001*
Stage group	*p<0.0001*
Vascular invasión	*p=0.259*
Capsule appearance	*p=0.003*
Edmondson	*p=0.053*
Steroid	*p<0.0001*
CNI	*p=0.041*
Location of recurrence	*p=0.622*

Tab. 5.7. The result of Multivariate test.

84 patients were died. And 34 caused by cancer(HCC), 6 patients died by cardiovascular diseases related with HCC (Tab. 5.8-9. And Figure. 5.19).

DEATH

		Frequency	Percentage	Percentage valid	Percentage accumulated
Valid	NO	163	66.0	66.0	66.0
	SI	84	34.0	34.0	100.0
	Total	247	100.0	100.0	

Tab. 5.8

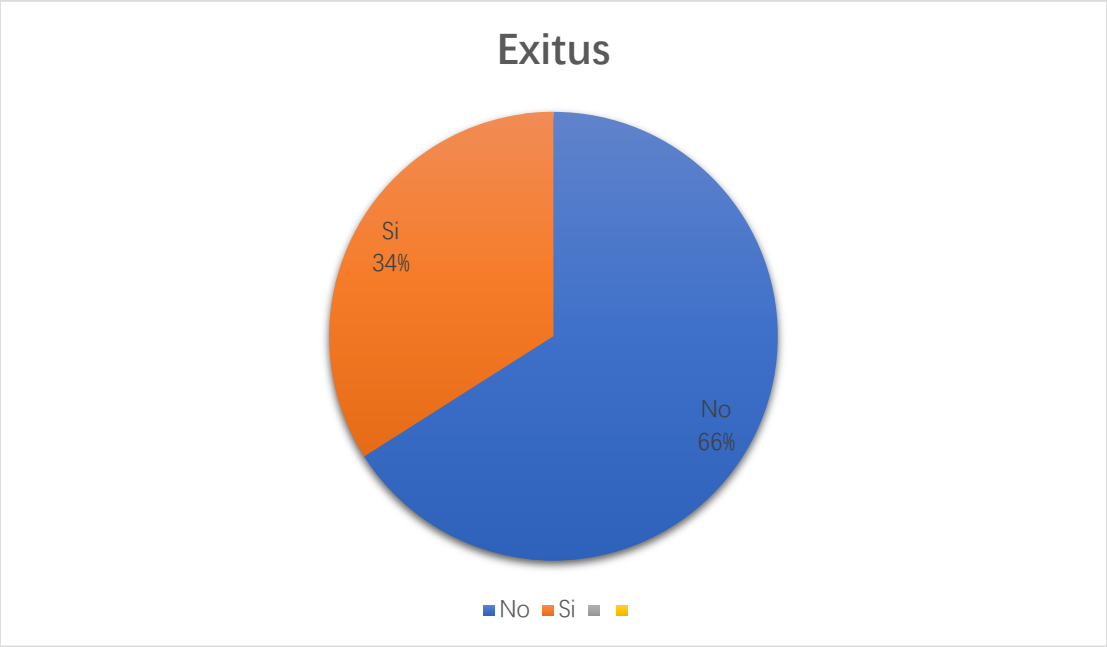


Figure. 5.20

CAUSE OF DEATH

		Frequency	Percentage	Percentage valid	Percentage accumulated
Valid		171	69.2	69.2	69.2
	CANCER	34	13.8	13.8	83.0
	POST-OLT	1	.4	.4	83.4
	CARDIOVASCULAR	6	2.4	2.4	85.8
	OTHERS	35	14.2	14.2	100.0
	Total	247	100.0	100.0	

Tab. 5.9

5.2. *De novo* neoplasia (NN)

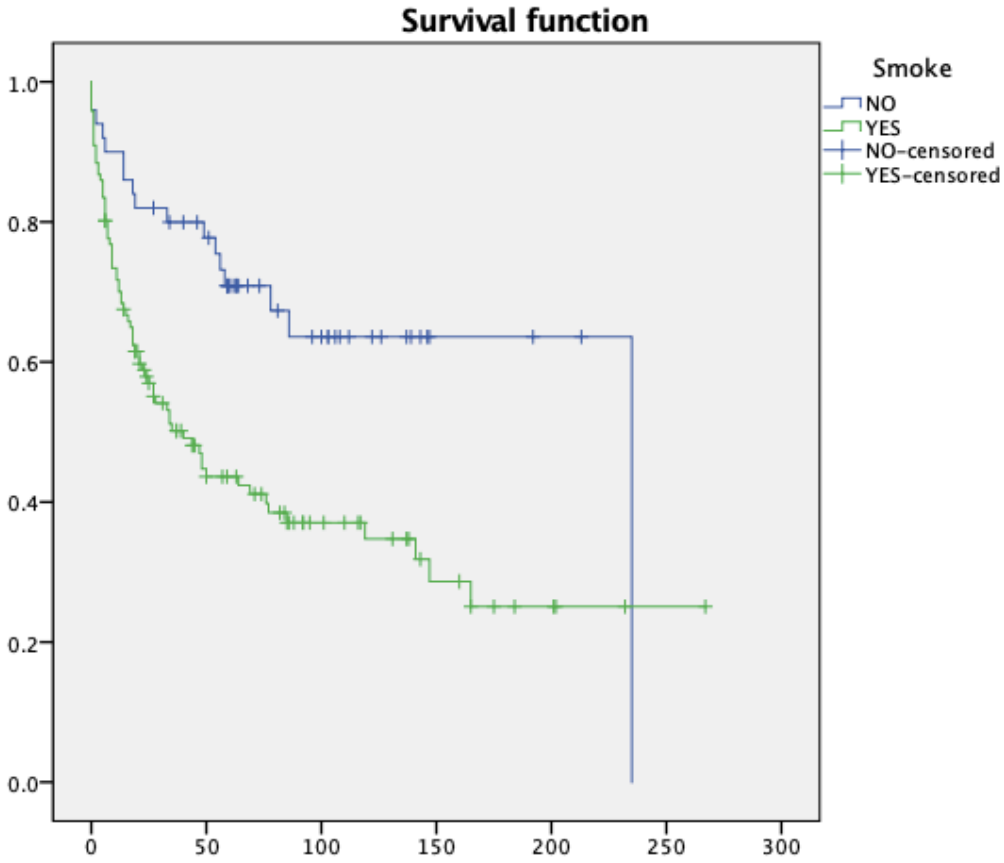


Figure 5.21. The survival rate of smoke in patients *de novo* neoplasia. (unit: month)

We use the test survival (Kaplan-Meier, with p=0.001) to check the related of smoke and survival time in patients de novo neoplasia. Different with related HCC and smoke, the smoke and the novo neoplasia is clearly that without smoke is much better than smoker.

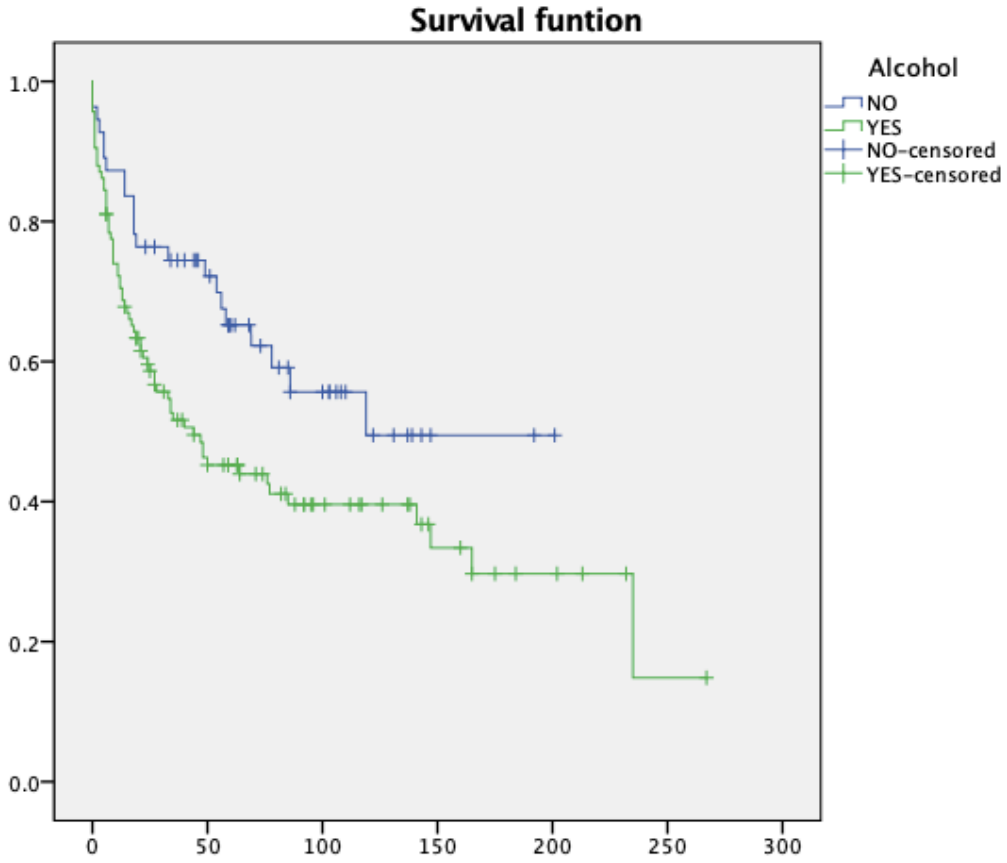


Figure 5.22. The survival rate of alcohol in patients with *de novo* neoplasia. (unit: month)

Use the test Kaplan-Meier, with the p=0.020. Intaking alcohol has bad effect for patients.

Tumor recurrence

		Frequency	Percentage	Percentage valid	Percentage accumulated
Valid	NO	102	59.6	59.6	59.6
	YES	69	40.4	40.4	100.0
	Total	171	100.0	100.0	

Table 5.10. Recurrent tumor in patients with *de novo* neoplasia

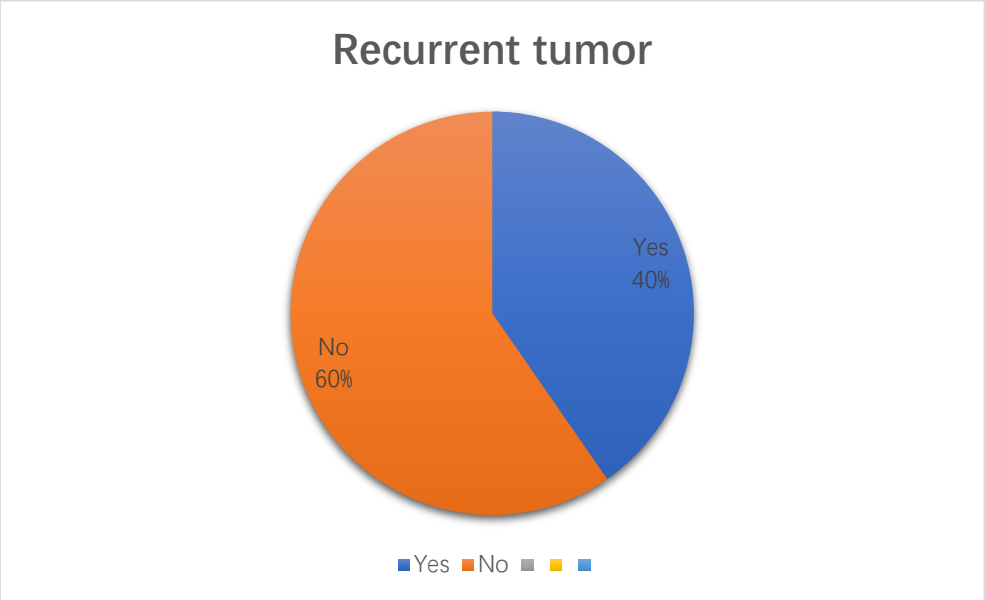


Figure 5.23. The distribution of recurrent tumor in the patients with NN.

In figure 5.24. we can observe the survival of patient in case of tumor recurrence in patients with NN.

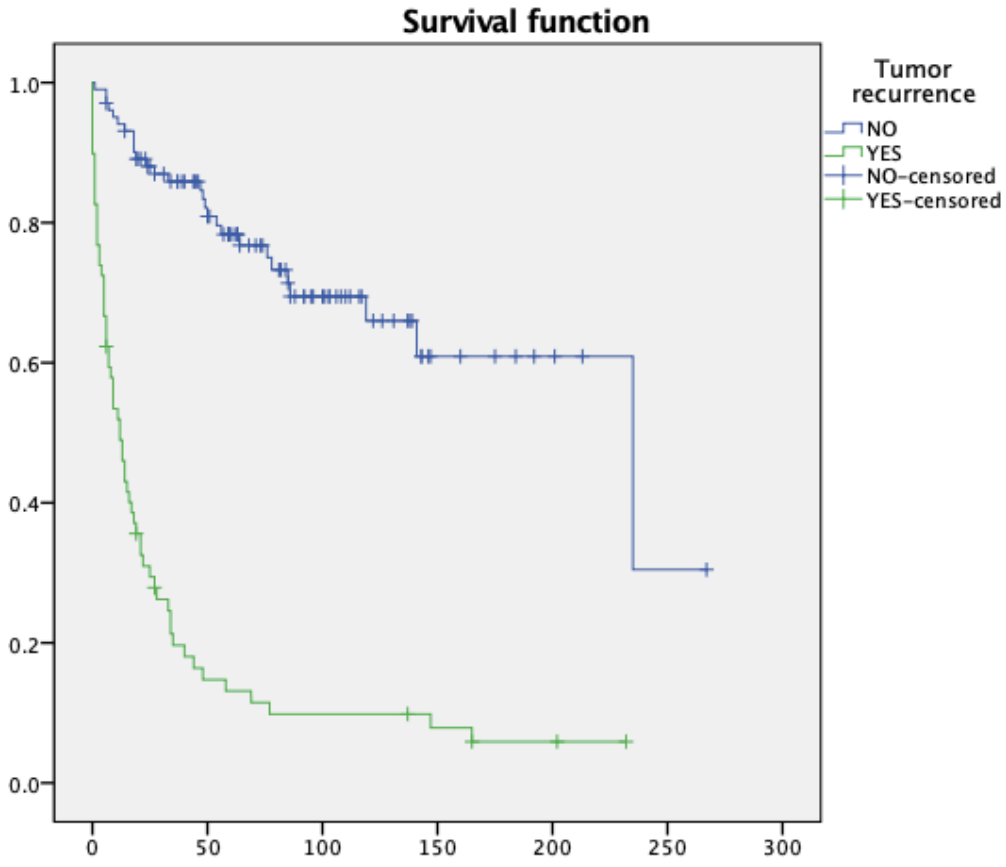


Figure 5.24. The survival rate of recurrent tumor in patients with NN. (unit: month)

There are 69 patients (40%) occur tumor recurrence. And use the survival test to check the related with recurrence and survival time, the Kaplan-Meier test ($p<0.0001$) shown the survival time. Without recurrence is more than 4 times than with it in the point 50 months. And in the point 200 months, without recurrence is almost 6 times than with it.

In next table we can see what treatment was offered to patients with NN: surgical resection or chemotherapy/radiotherapy.

Treatment way		
Type of treatment	Frequency	Percentage
Resection	89	52.05%
QT or RT	47	27.49%
No treatment	35	20.46%
Total	171	100%

Table 5.11. The frequency and percentage of treatment way in patients with NN.

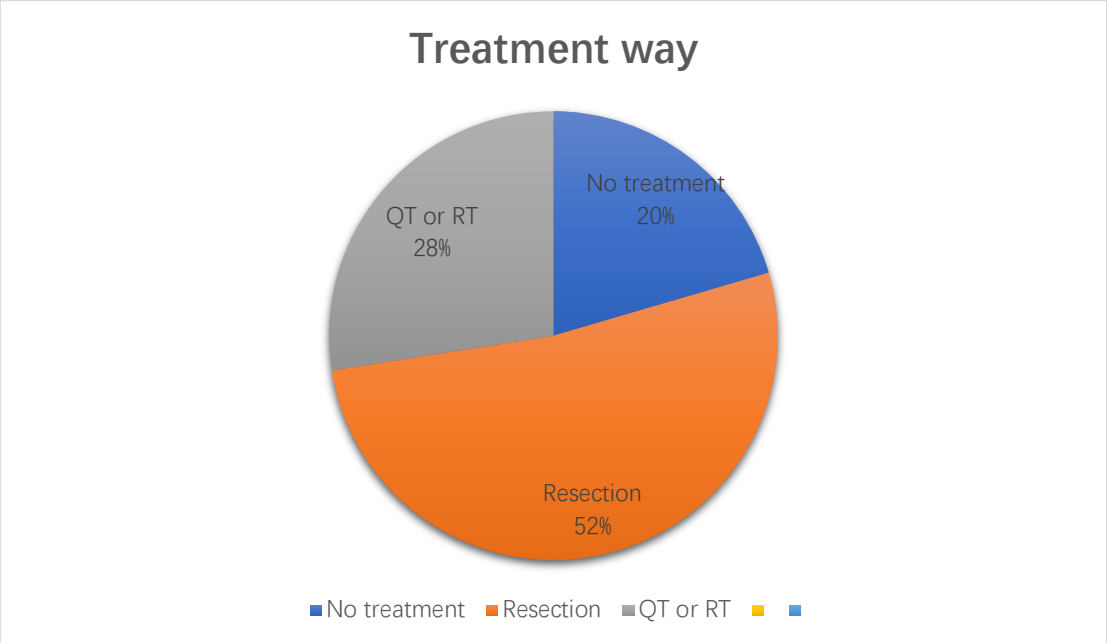


Figure 5.25. The distribution of treatment way (QT-RT means: Chemotherapy or Radiotherapy)

We have studied (figure 5.26) the survival of patients with NN in case of treatment way (QT-RT means: Chemotherapy or Radiotherapy).

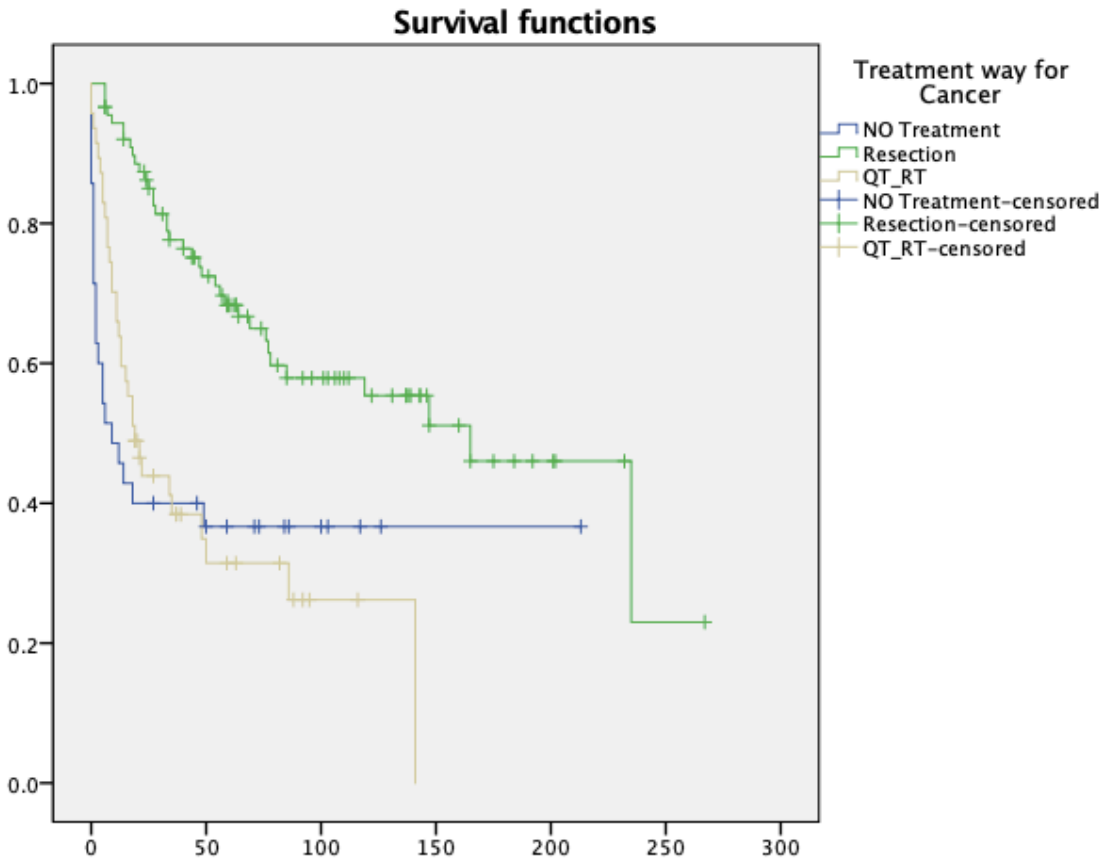


Figure 5.26. The survival rate of the treatment way in patients with NN. (unit: month)

In the table 2 and figure 5, described the distribution of treatment way in patients with NN. With the survival test ($p<0.0001$), the resection is the best way to treatment tumor in NN. Chemotherapy or Radiotherapy and without treatment is similar, but Chemotherapy or Radiotherapy is little better than without treatment in the short-term.

In next figure we can observe survival of transplanted patients with NN in function of tumor size (T stage).

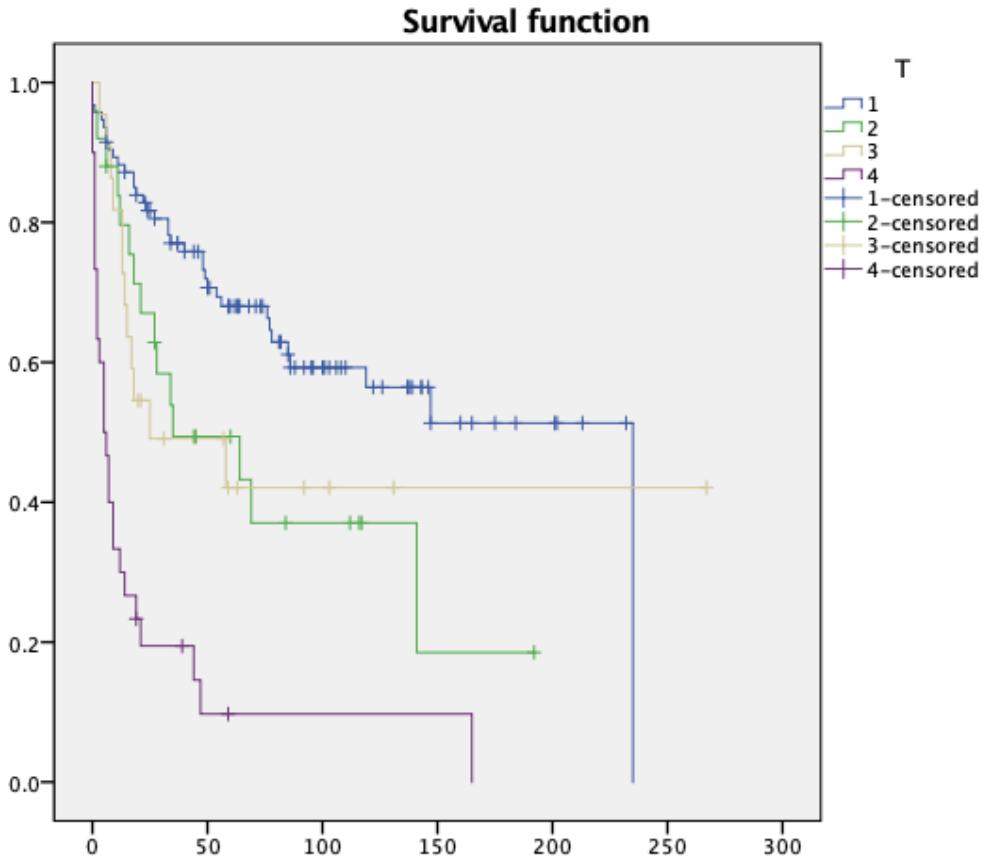


Figure 5.27. The survival rate of Stage T in patients with NN. (unit: month) ($p<0.0001$)

Obviously, the survival time of stage 1 of T is best. As we know, the stage 4 is severe, so the worst is stage 4, the stage 2 and stage 3 are similar but the stage 2 is better than stage 3.

Next figure shows the same in case of metastatic nodes (N stage) and distance metastasis (M stage).

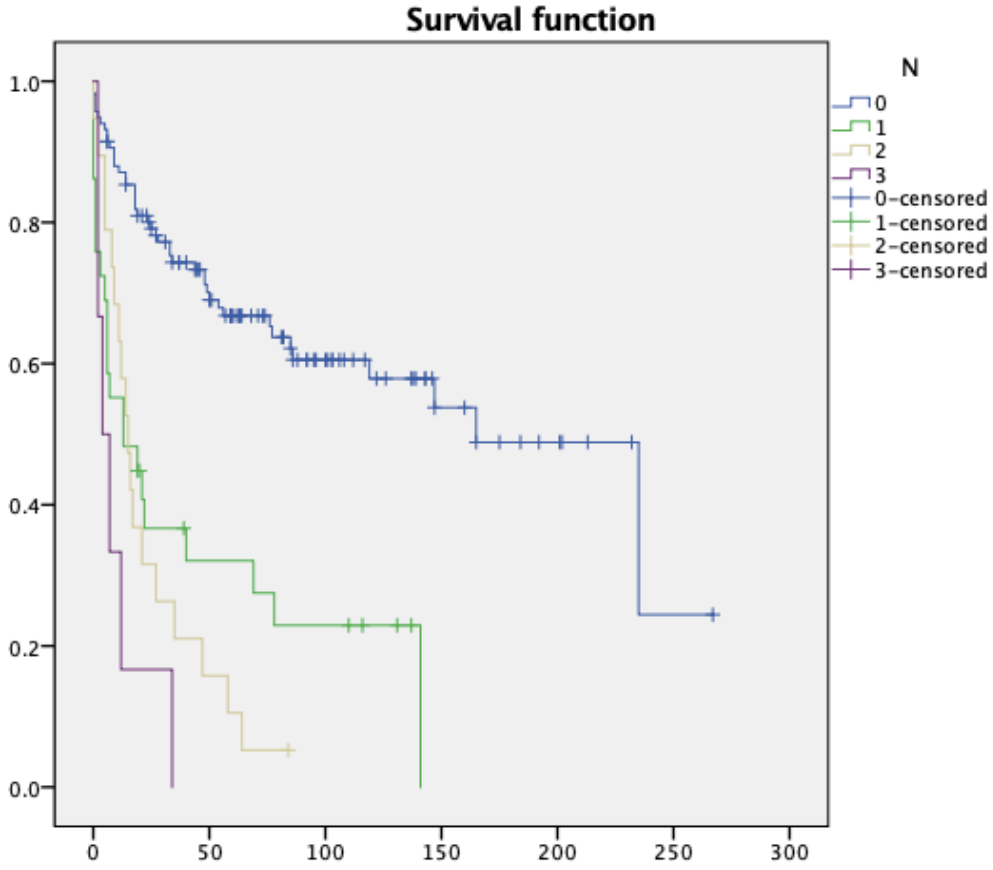


Figure 5.28. The survival rate of Stage M in patients with NN. (unit: month)

($p<0.0001$)

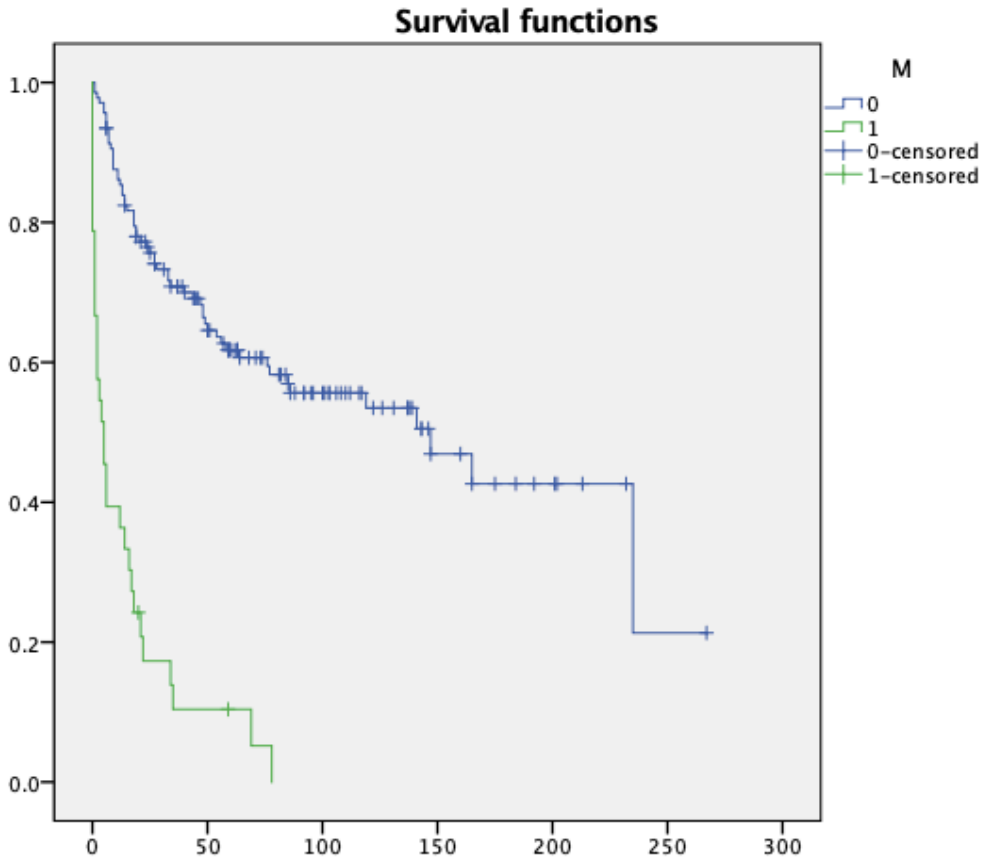


Figure 5.29. The survival rate of Stage M in patients with NN. (unit: month) ($p<0.0001$)

The figure 5.28. is shown clearly that stage 0 of N is the best. And the figure 5.29. show the without metastasis distal, the survival time is larger.

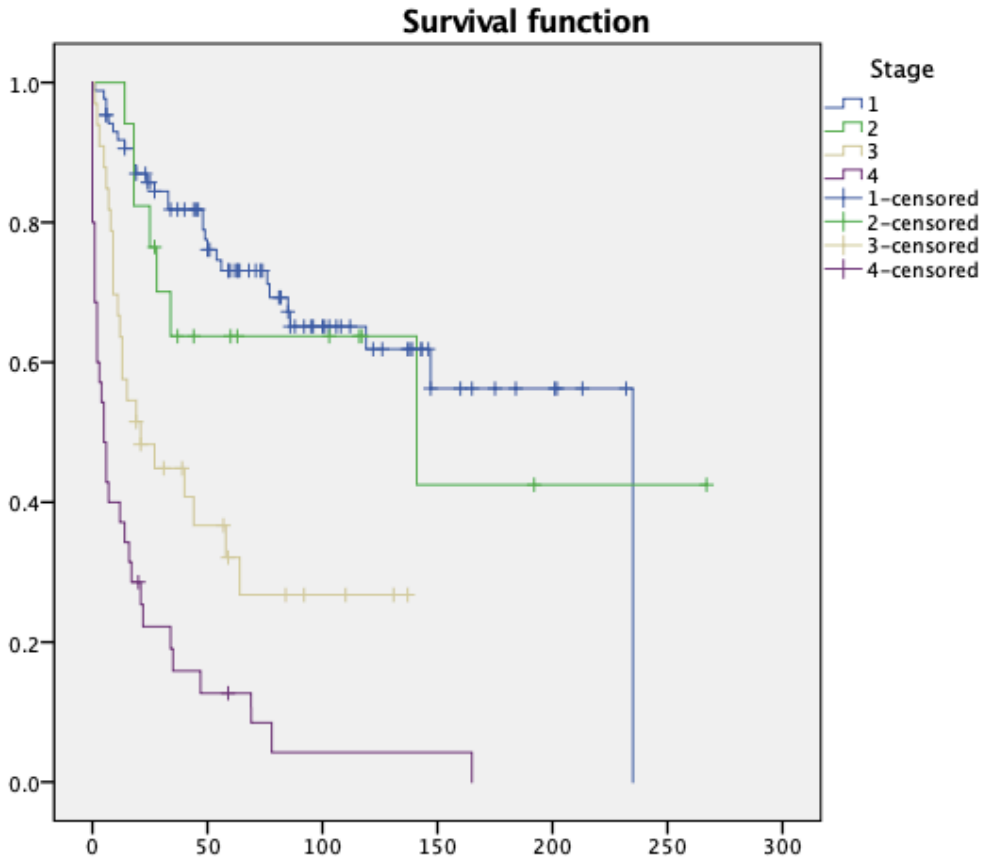


Figure 5.30. The survival rate of Stage group in patients with NN. (unit: month) ($p<0.0001$)

In the general, the stage 1 is better than others, although the rate of stage 2 higher than stage 1 twice.

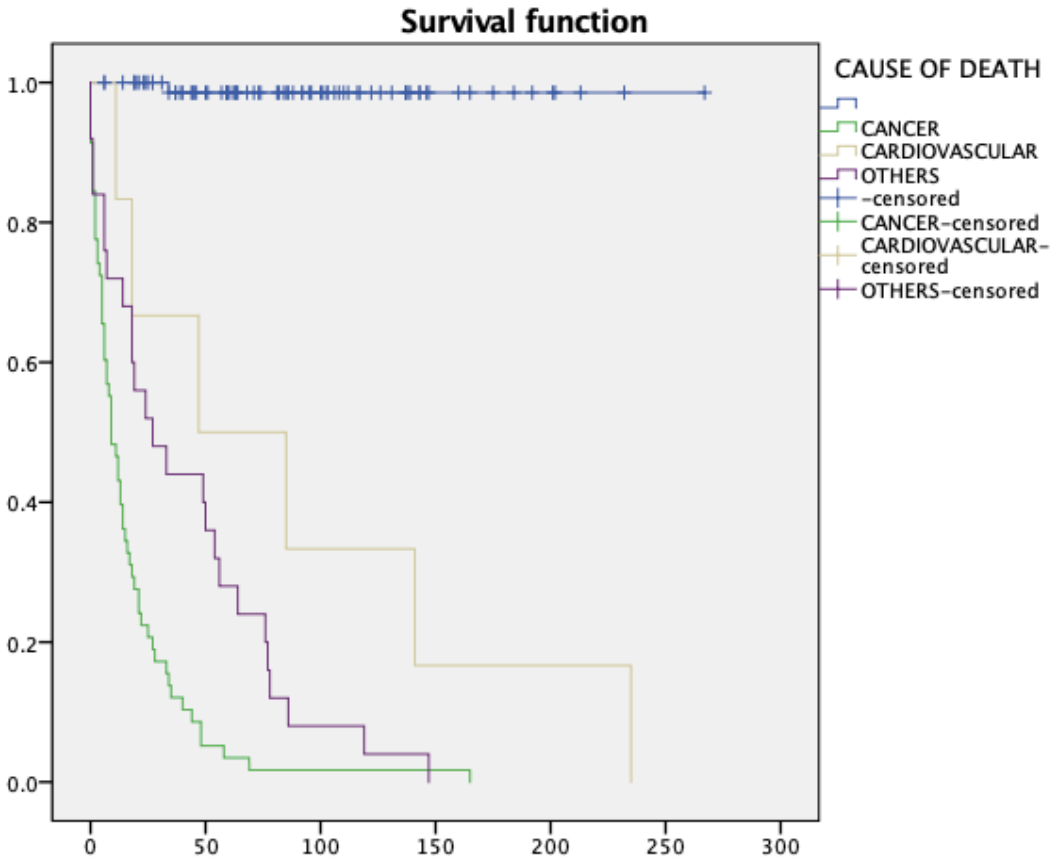


Figure 5.31. The survival rate of died cause in patients with NN. (unit: month), ($p<0.0001$)

The blue line is the rate of patients alive. There are 25 patients died by other cause, 6 patients by cardiovascular and 58 patients by cancer. Cancer kills patients before than cardiovascular diseases.

mTORi-CNI in NN		
Type	Frequency	Percebtage
mTORi	77	45.00%
CNI	69	40.40%
MMF	25	14.60%

Table 5.12. The distribution of inhibitor used in patients with NN

In next figure we can observe the survival of patients with NN depending of immunosuppressant treatment.

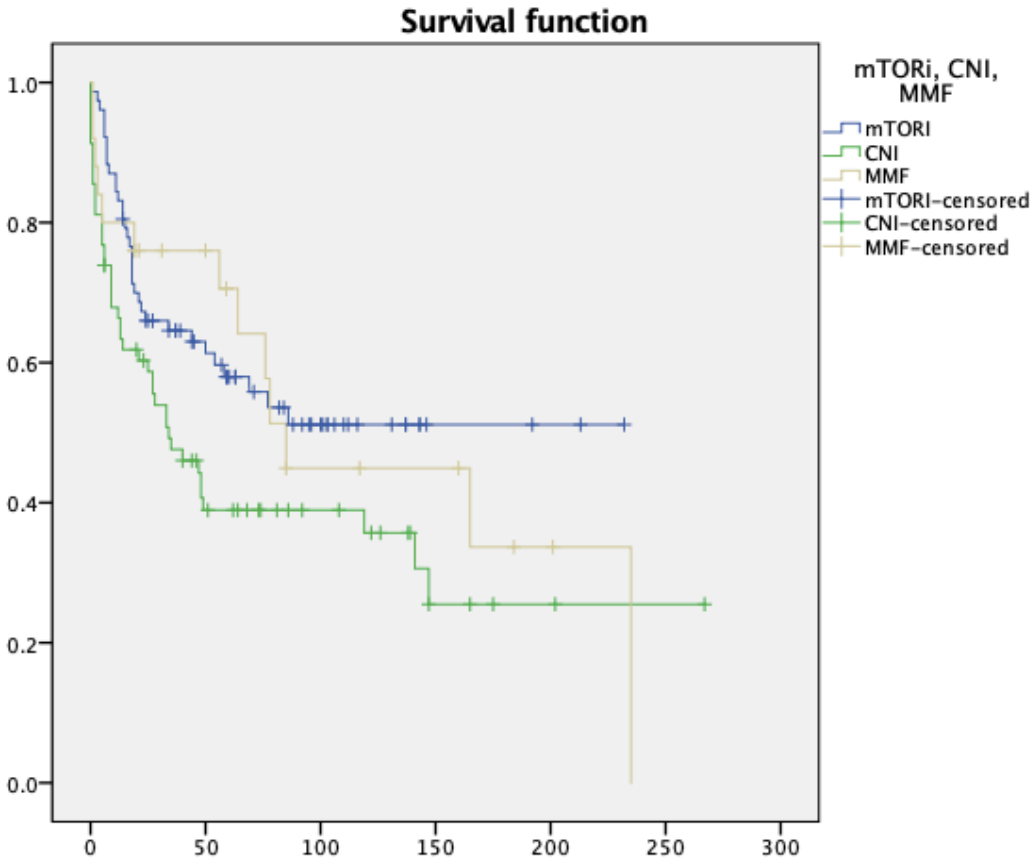


Figure 5.32. The survival rate of CNI, mTORi and MMF in patients with NN. (unit: month)

With the p=0.058, the survival test shown that mTORi and MMF are better than CNI. In the short-term, MMF is similar with CNI, and mTORi is better than others two. In the middle-term, the MMF is the best in these 3 inhinitor, however, later than 80 months, the effect of MMF is worse than mTORi. CNI is the worst in these 3 treatment.

Sirolimus VS Everolimus		
Type of mTORi	Frequency	Percentage
Sirolimus	54	70.13%
Everolimus	23	29.87%
Total	77	100.00%

Table 5.13. The mTORi distribution in patients with NN

In next figure (figure 5.32.), we show the survival in case of type of mTORi in patients with NN.

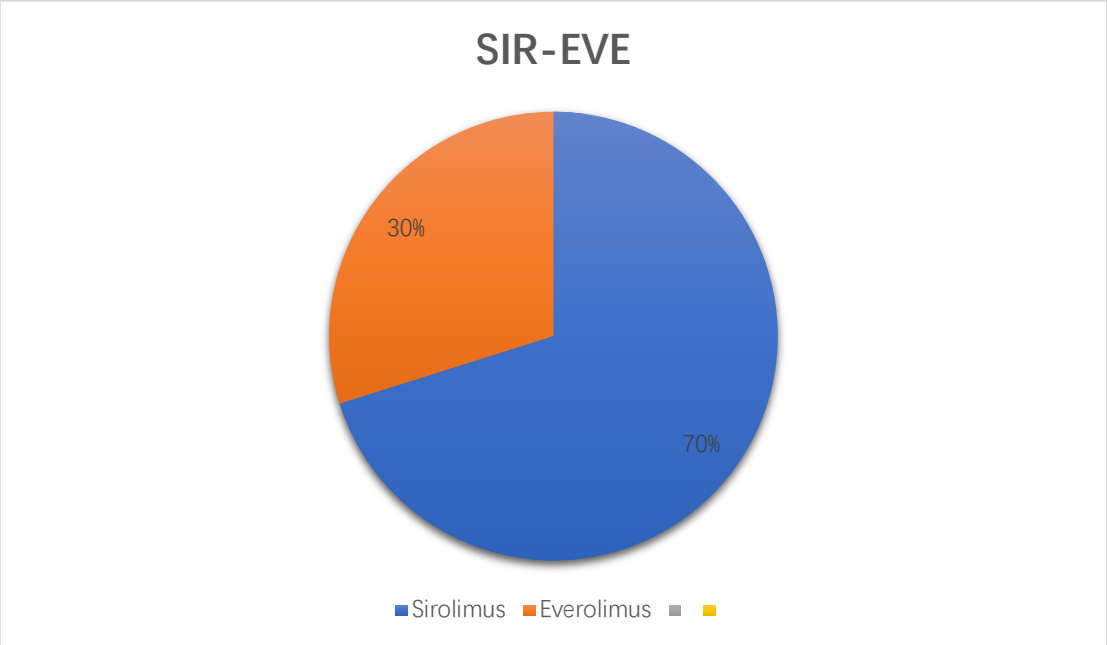


Figure 5.33. The distribution of mTORi in the patients with NN.

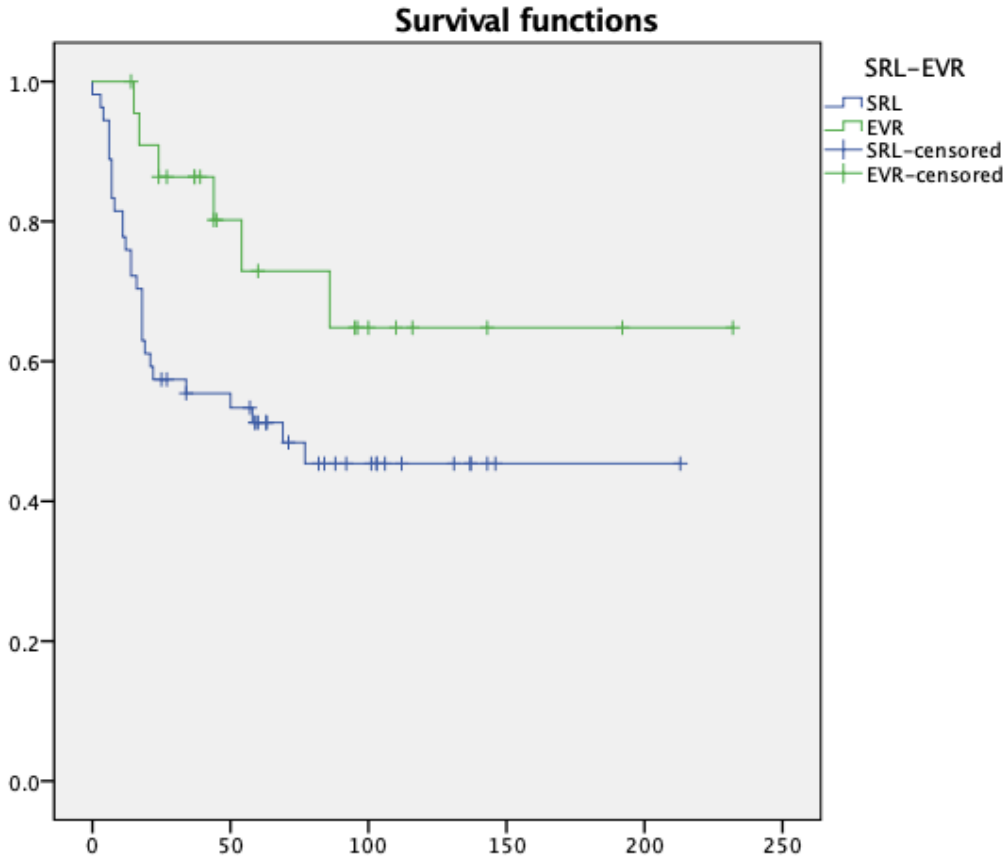


Figure 5.34. The survival rate of mTORi in patients with NN. (unit: month)

Compare in these two inhibitor. Due to the Kaplan-Meier test (with the p=0.042), the effect of Everolimus is better than Sirolimus. And theirs different is about 10%.

Skin cancer

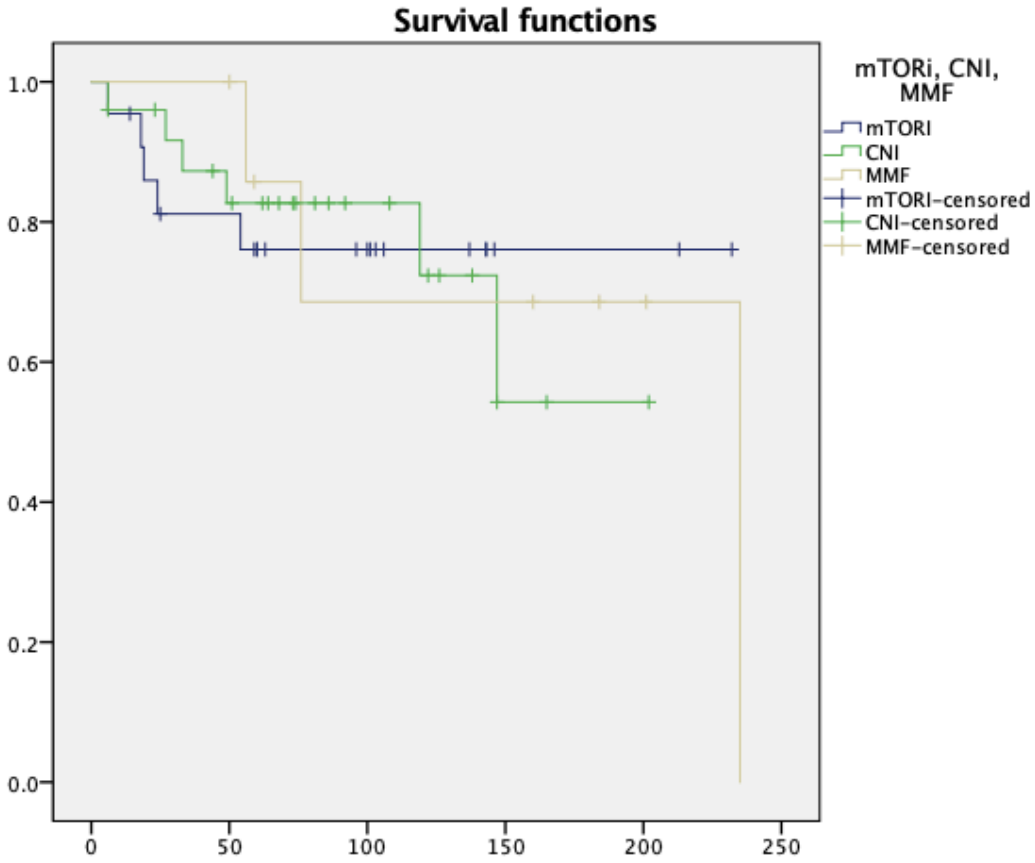


Figure 5.35. The survival rate of CNI, mTORi and MMF used in patients with skin cancer. (unit: month) (p=0.945)

For the skin cancer, the advantage of these 3 is not clearly, in the first 50 months, the MMF is better than others two; between the 75 months and 120 months, the CNI is better than others; and after 120 months, the mTORi shown better effect.

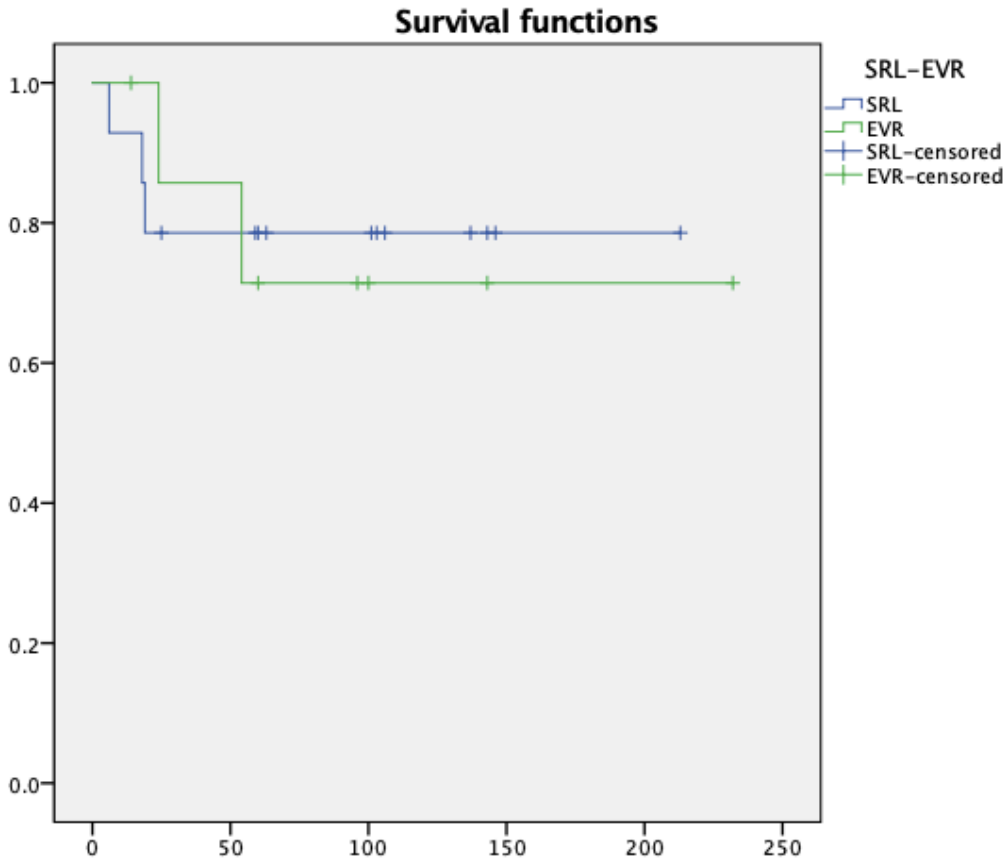


Figure 5.36. The survival rate of Sirolimus and Everolimus in patients with skin cancer. (unit: month) (p=0.886)

There is no difference in survival of skin cancer between Everolimus or Sirolimus ($p > 0.05$)

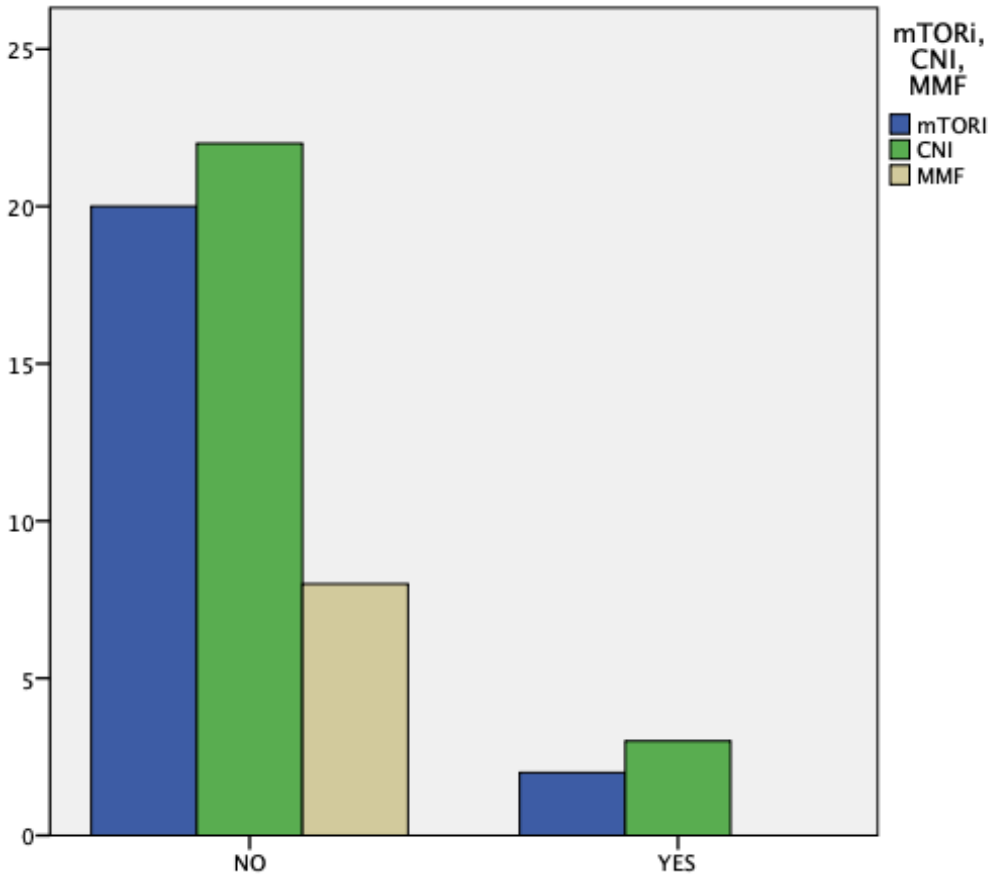


Figure 5.37. The distribution of patient used immunosuppressant with or without tumor recurrence.(p=0.590)

There are 22 patients with skin cancer used immunosuppressant mTORi, the survival of with tumor recurrence is higher. But only 2 patients with tumor recurrence, and this test dose not has statistical significance. (p=0.440)

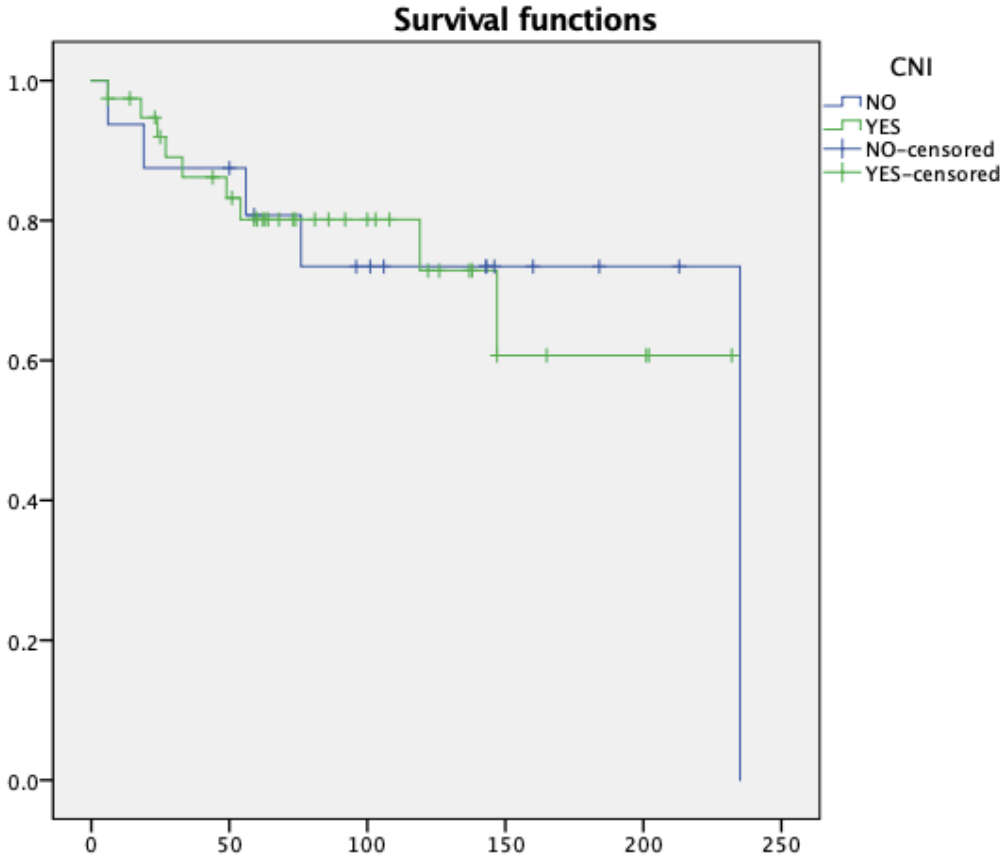


Figure 5.38. The survival rate of CNI used in patients with skin cancer. (unit: month) (p=0.883)

Also, there is no difference in survival in case of CNI treatment.

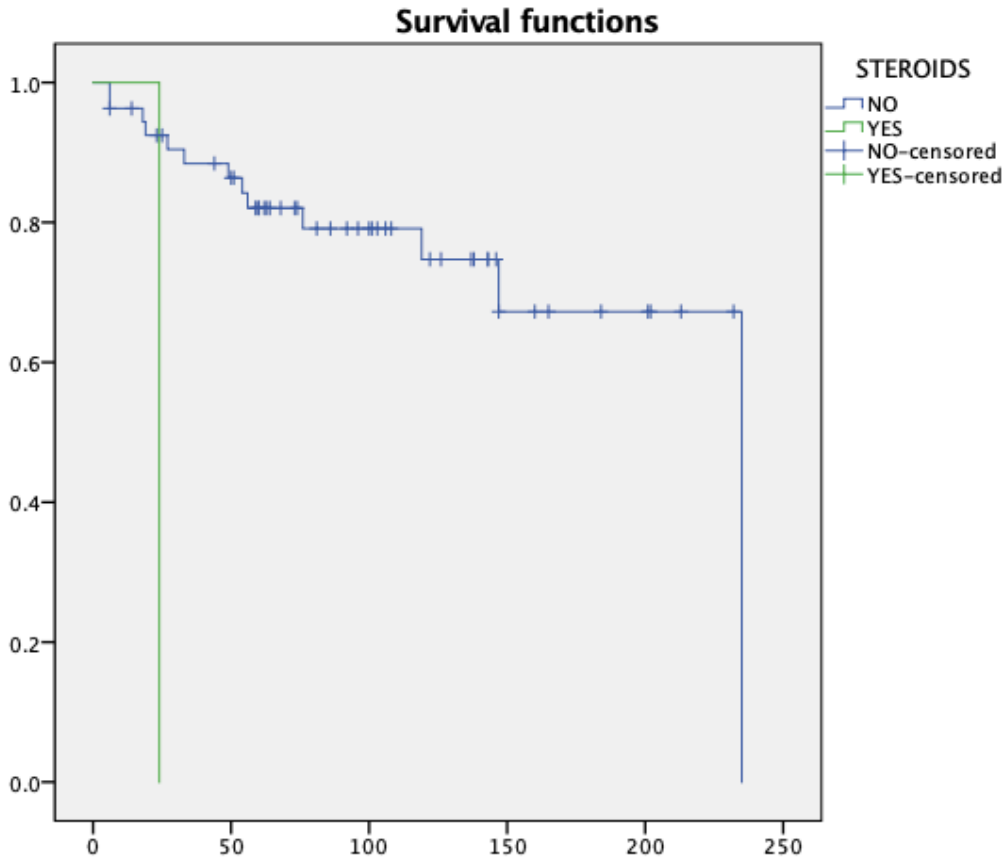


Figure 5.39. The survival rate of steroid used in patients with skin cancer. (unit: month) (p=0.003)

Without steroid therapy is much better than patient with the steroid therapy.

ORL cancer

In next figure we can observe survival of ORL cancer patients depending of treatment used, surgical resection VS chemotherapy/radiotherapy.

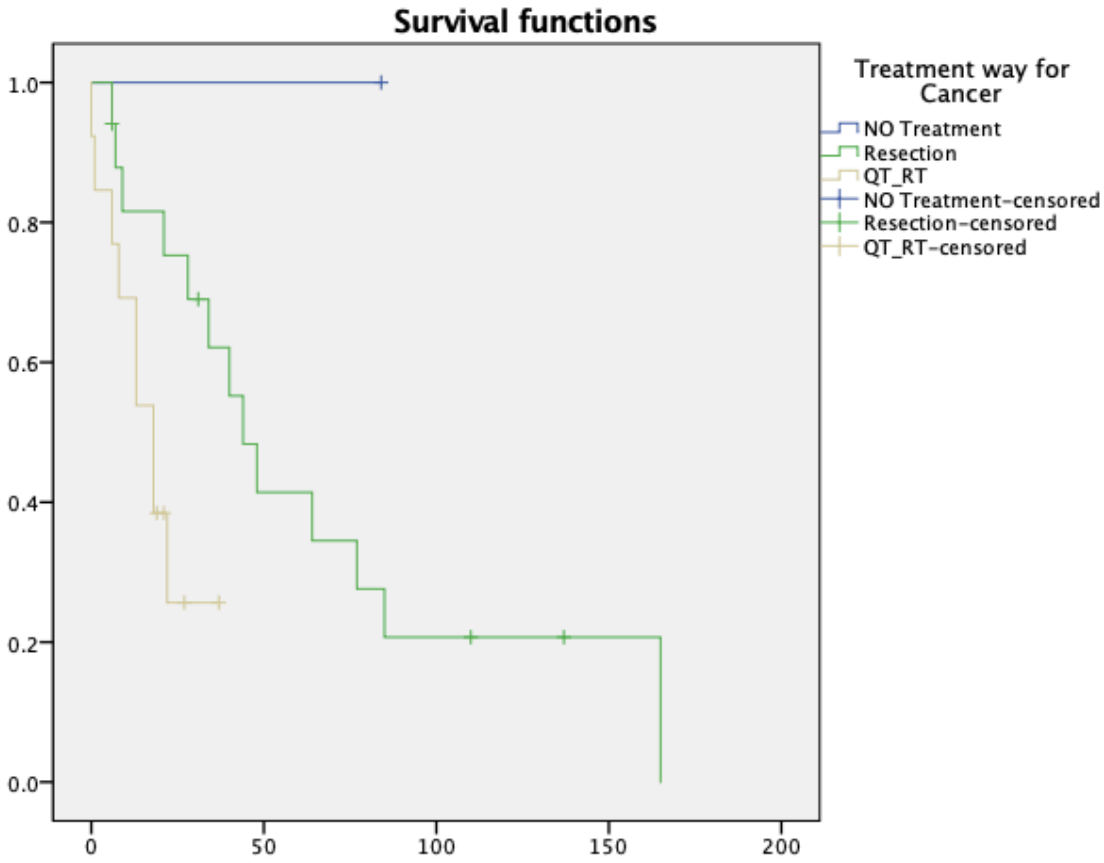


Figure 5.40. The survival rate of treatment way in patients with Otorhinolaryngologic cancer. (unit: month) (p=0.030)

As we can observe, surgical resection gives a better survival in patients with ORL cancer.

Next figure shows survival in function of immunosuppressant regimen we used in these patients, mTORi, CNI or MMF.

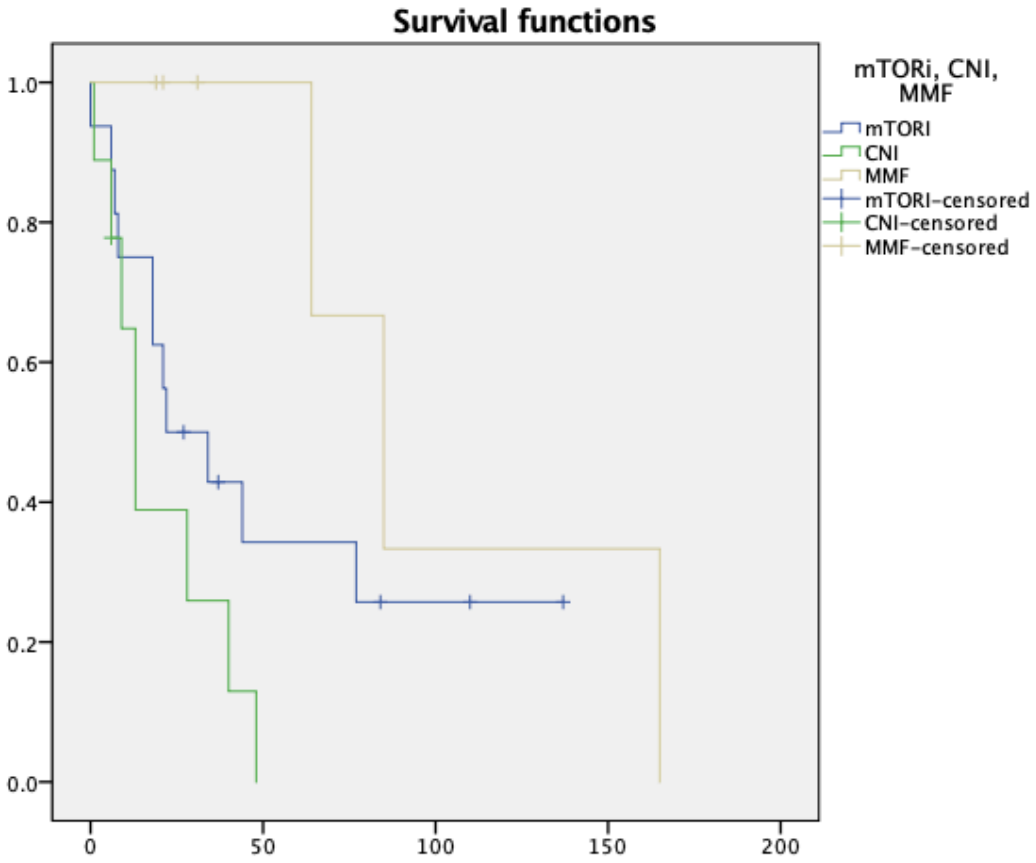


Figure 5.41. The survival rate of CNI, mTORi and MMF used in patients with Otorhinolaryngologic cancer. (unit: month) (p=0.018)

The MMF treatment is better than mTORi and CNI. Also, mTORi is better than CNI. ($p<0.05$)

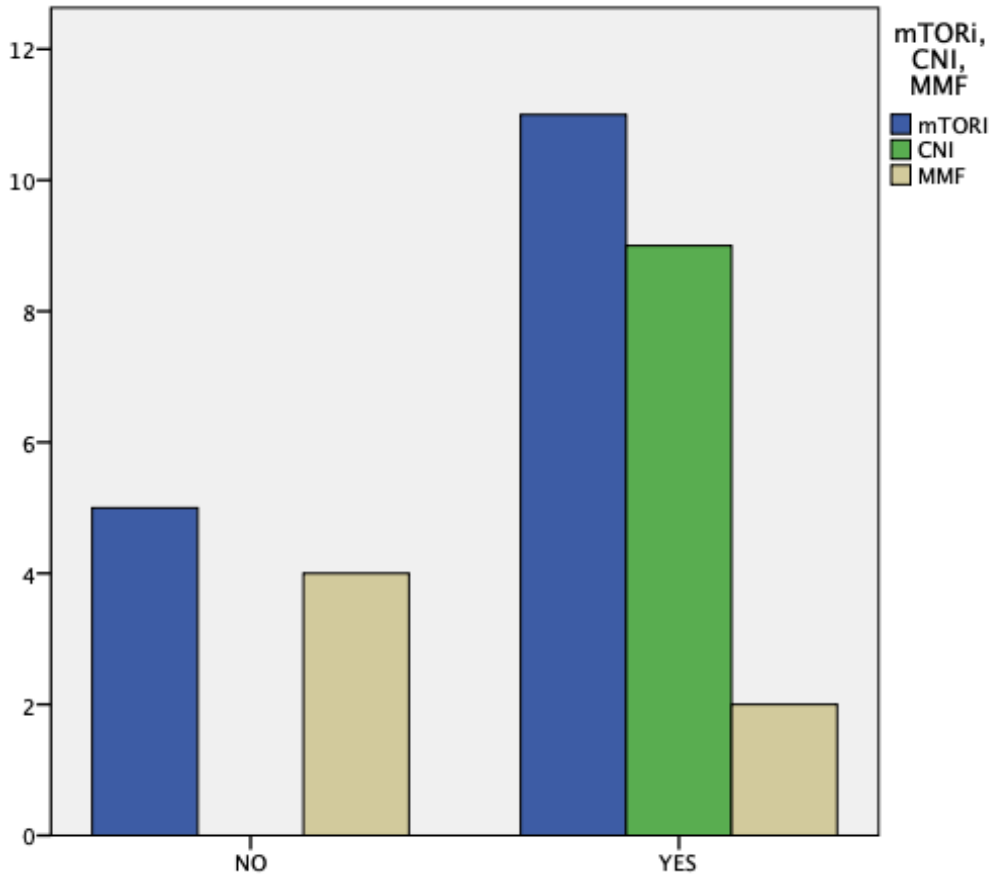


Figure 5.42. The distribution of patient used immunosuppressant with or without tumor recurrence.

There are 22 patients without tumor recurrence (11 patients with mTORi, 9 patients with CNI and 2 patients with MMF), and 9 patients with tumor recurrence. (p=0.020)

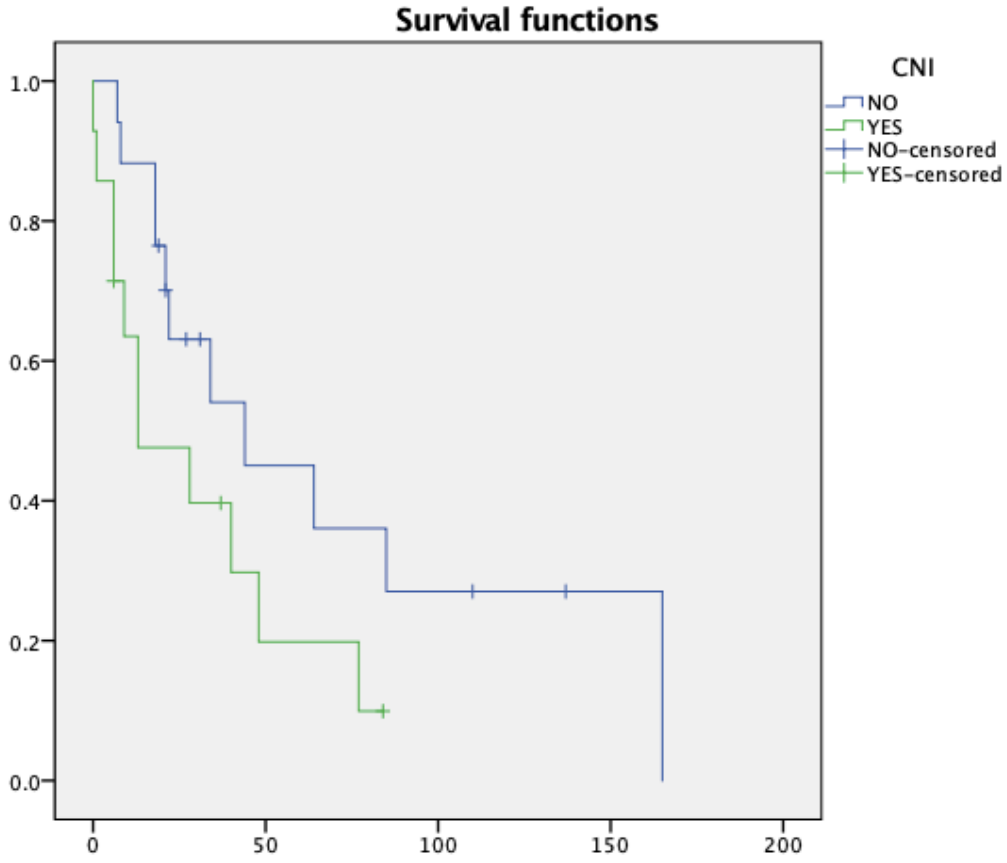


Figure 5.43. The survival rate of CNI used in patients with Otorhinolaryngologic cancer. (unit: month) (p=0.086)

Without CNI's therapy is better than CNI therapy.

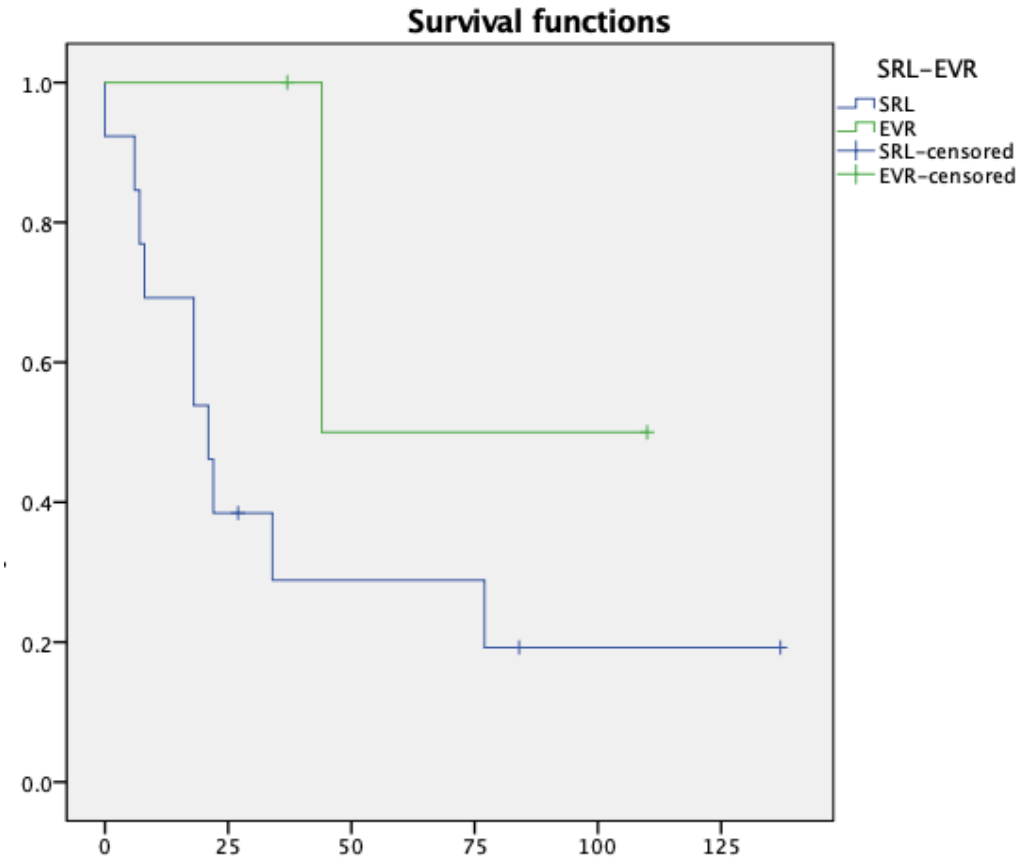


Figure 5.44. The survival rate of CNI used in patients with Otorhinolaryngologic cancer. (unit: month) (p=0.159)

Everolimus seems to be better than Sirolimus, but it is only a clinical observation without statistical significance. (p>0.05)

Lung cancer

In figure 5.45. we can see survival of lung cancer depending no treatment offered: palliative, chemotherapy/radiotherapy or surgical resection.

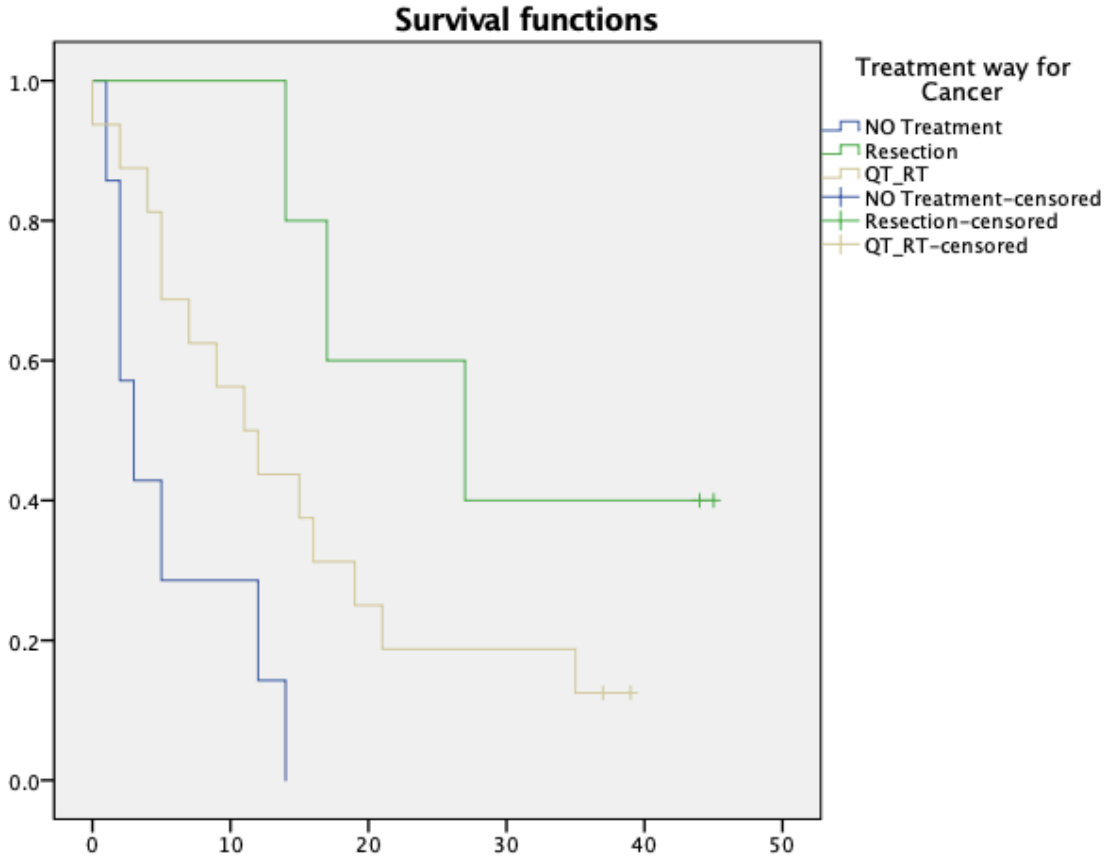


Figure 5.45. The survival rate of treatment way in patients with lung cancer. (unit: month) (p=0.005)

The resection is the best than others 2 treatment way. Without treatment is the worst way, and cannot reach 15 months.

Next figures show survival of patients with lung cancer depending of inmunosupressant regimen.

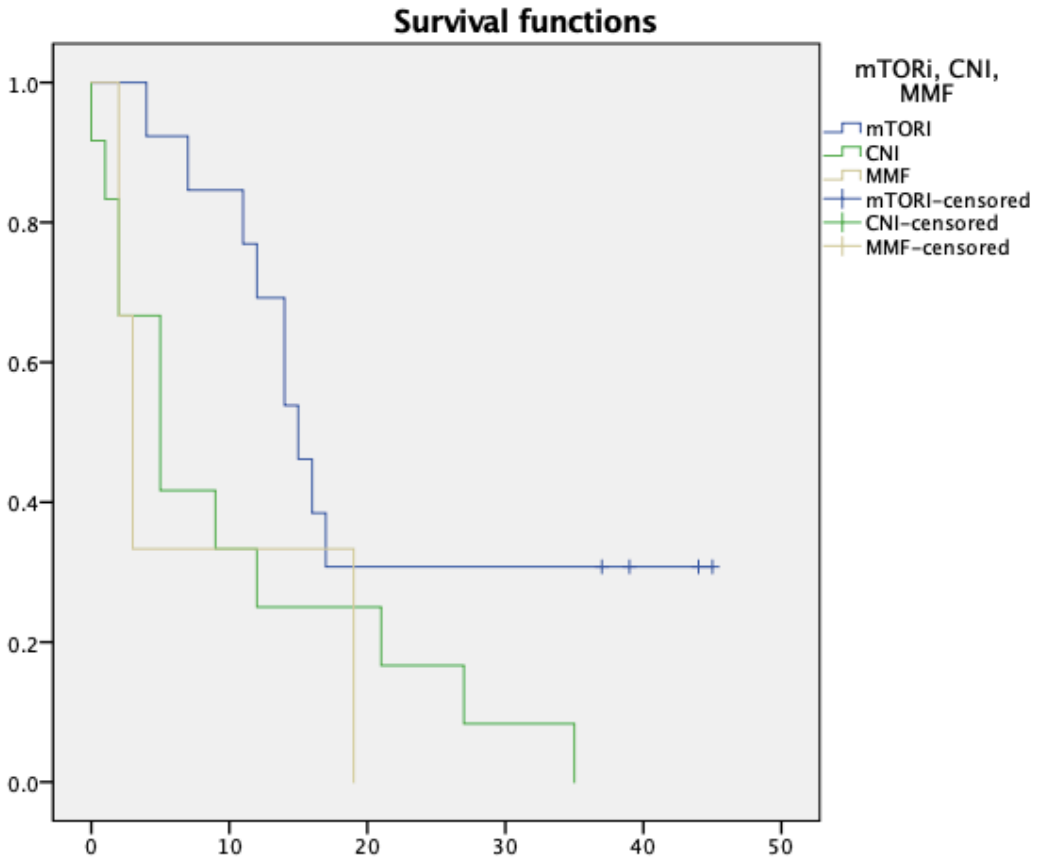


Figure 5.46. The survival rate of CNI,mTORi and MMF used in patients with lung cancer. (unit: month) (p=0.067)

mTORi is better than others 2 drugs CNI and MMF, although it is only a clinical observation with a little statistical signification (p<0,05). Survival time is largest that almost reach 50 months.

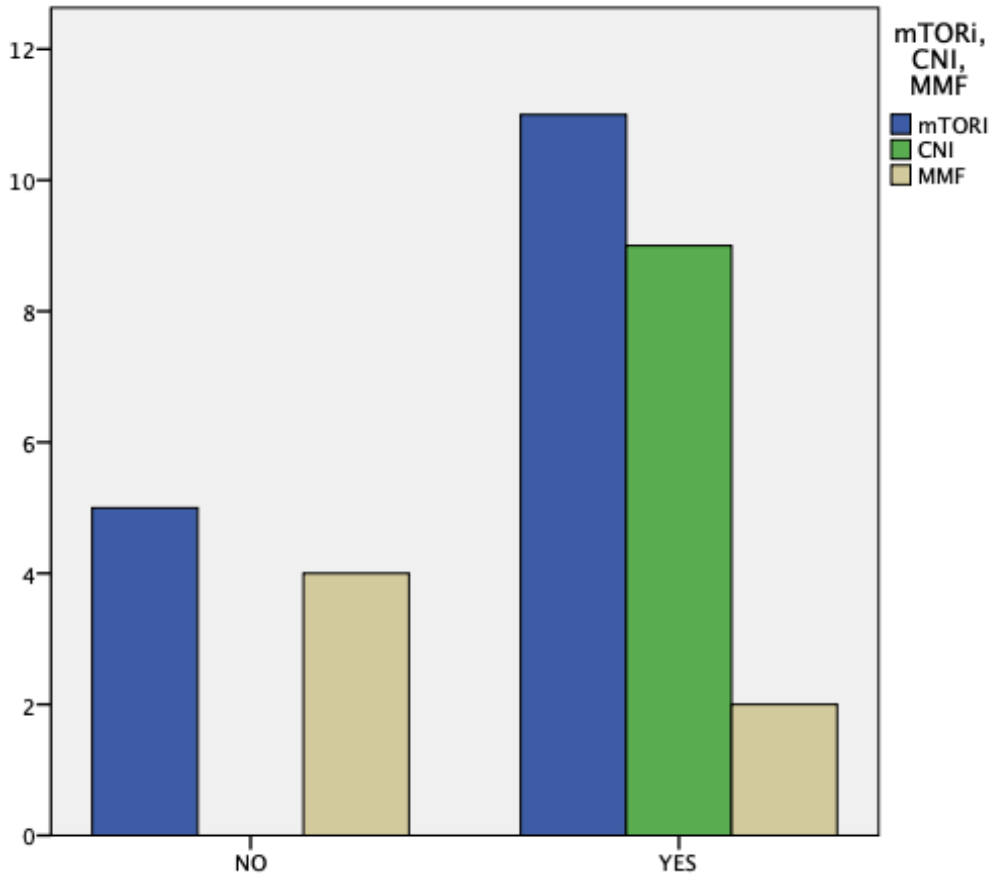


Figure 5.47. The distribution of patient used immunosuppressant with or without tumor recurrence.

There are 23 patients with tumor recurrence, 9 patients with mTORi regimen, 11 patients with CNI regimen and 3 patients with MMF regimen. (p=0.238)

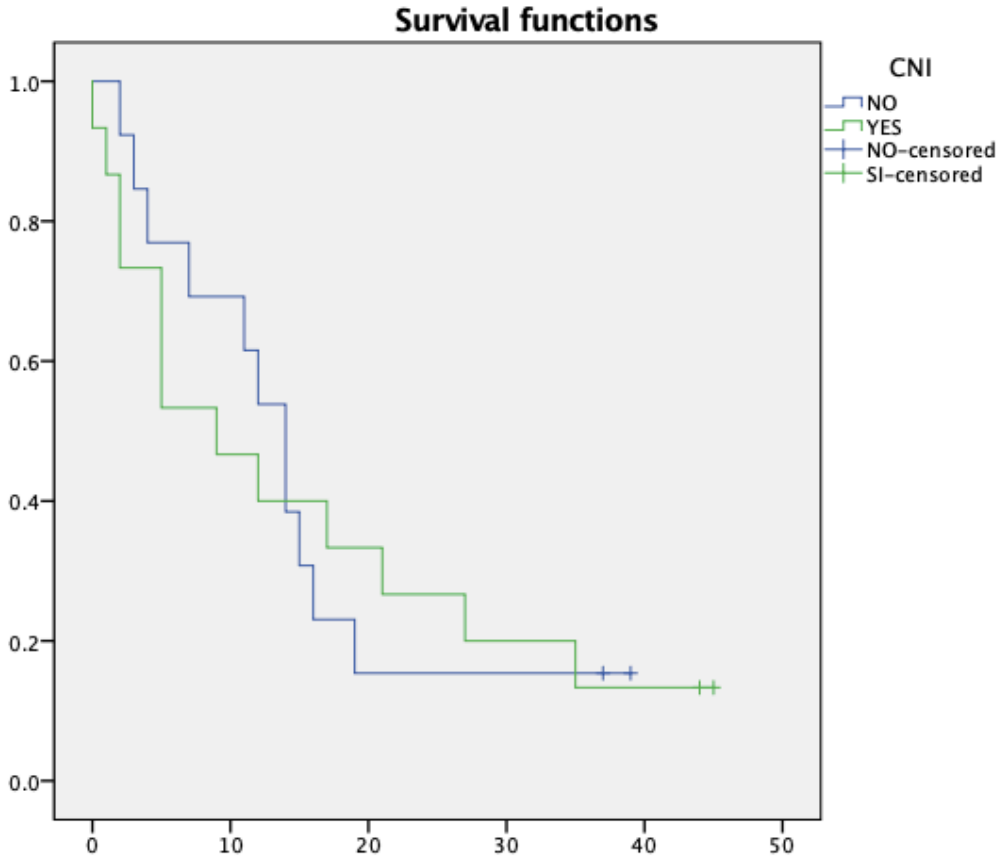


Figure 5.48. The survival rate of CNI used in patients with lung cancer. (unit: month) (p=0.910)

There is no difference if we use or not use CNI in inmunosupressant regimen

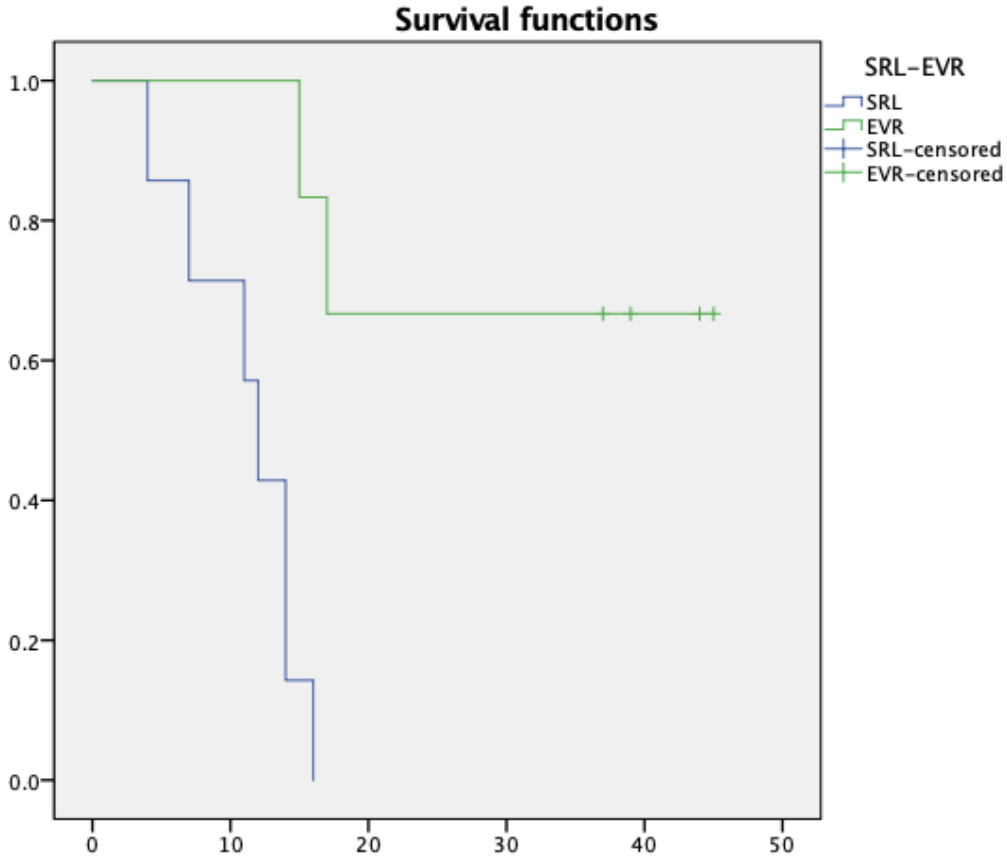


Figure 5.49. The survival rate of mTORi used in patients with lung cancer. (unit: month) (p=0.001)

There are 14 patients with lung cancer use mTORi, and with the figure 28. shown, Everolimus is much better than Sirolimus. Especially the survival time, with everolimus therapy is more than 2 times than patients with sirolimus.

Digestive cancer

In next figures we can observe survival of patients with digestive cancer in function of immunosuppressant treatment we used. Due to the little number of patients with digestive cancer (colorectal, stomach, esophagus) (n=14), we make the whole digestive system cancer together to make the test survival (Kaplan-Meier).

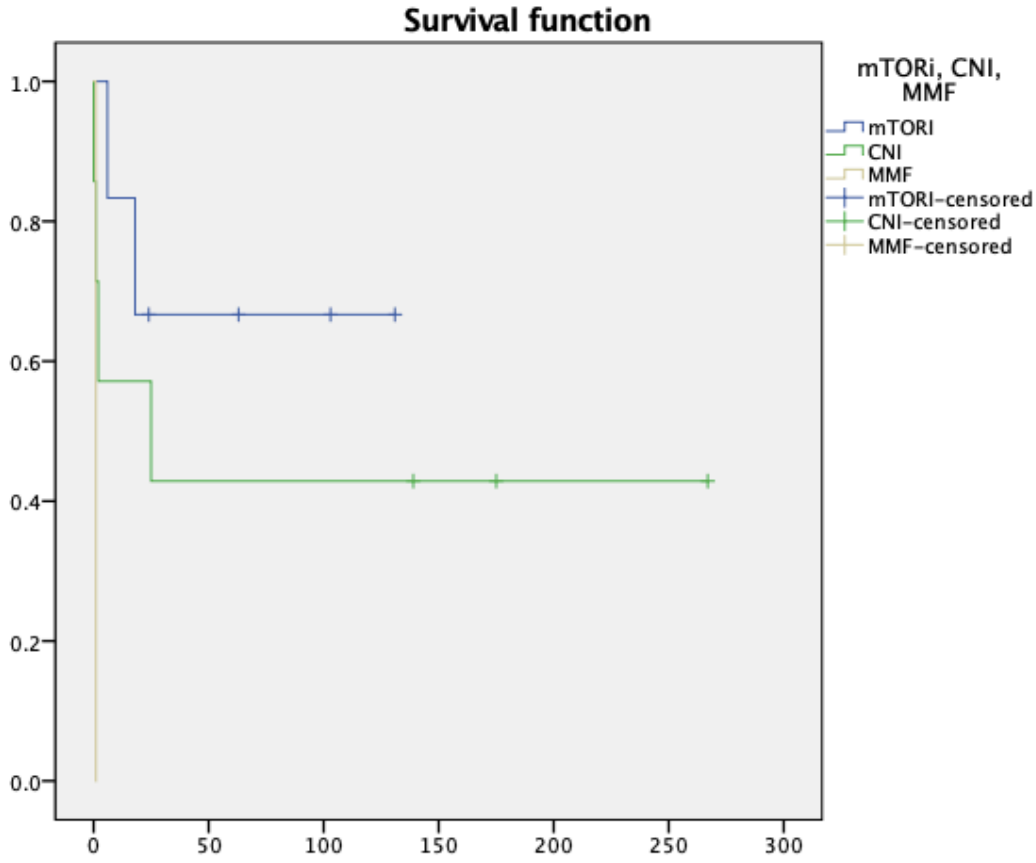


Figure 5.50. The survival rate of inhibitor used in patients with digestive system cancer. (unit: month) (p=0.150)

mTORi's efficacy is the best of these 3 drugs.

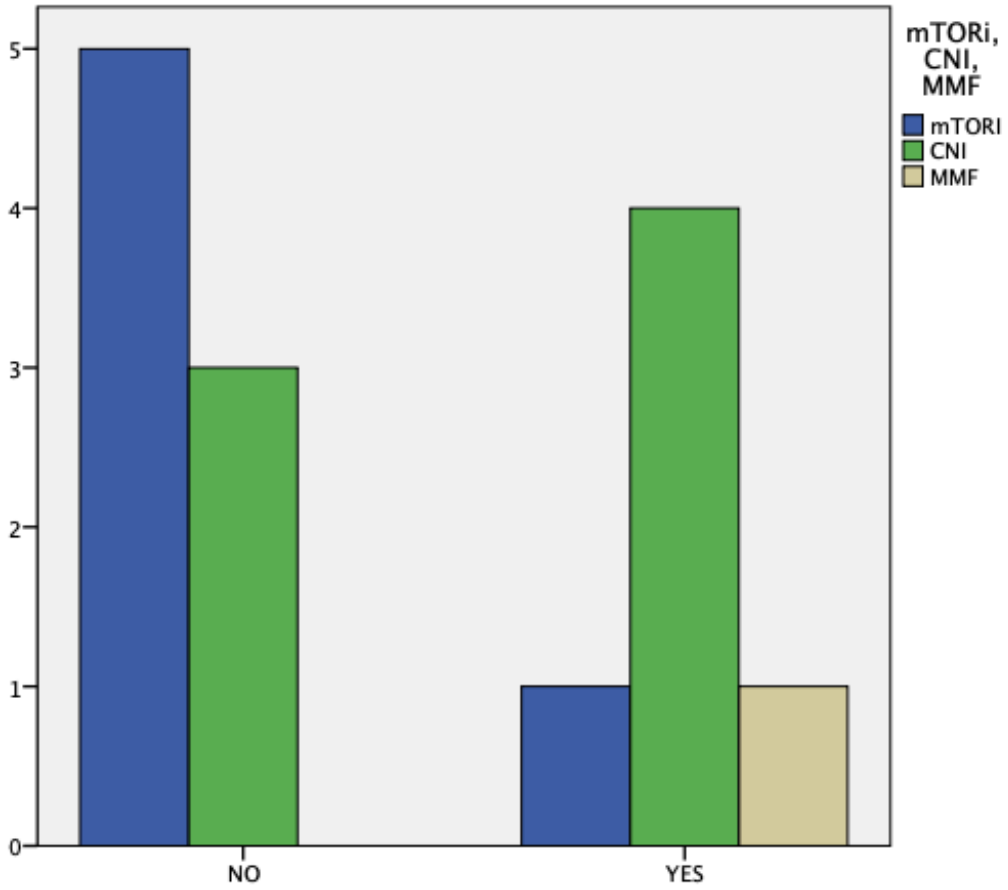


Figure 5.51. The distribution of patient used immunosuppressant with or without tumor recurrence.

There are 6 patients with tumor recurrence (1 patient with mTORi, 4 patients with CNI and 1 patient with MMF), and 8 patients without tumor recurrence. (p=0.166)

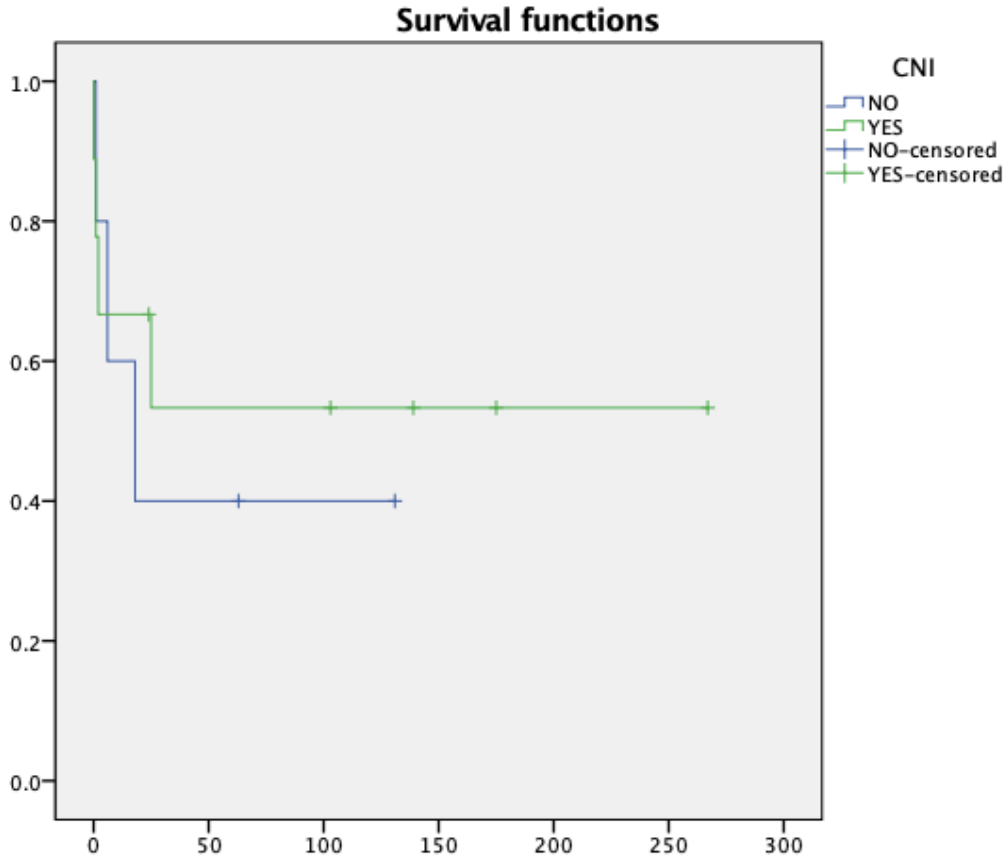


Figure 5.52. The survival rate of CNI used in patients with digestive system cancer. (unit: month) (p=0.694)

There is no difference in survival using or not CNI treatment.

Because of all patients with digestive system cancer do not use the steroid, this survival test not present the compared of with and without steroid.

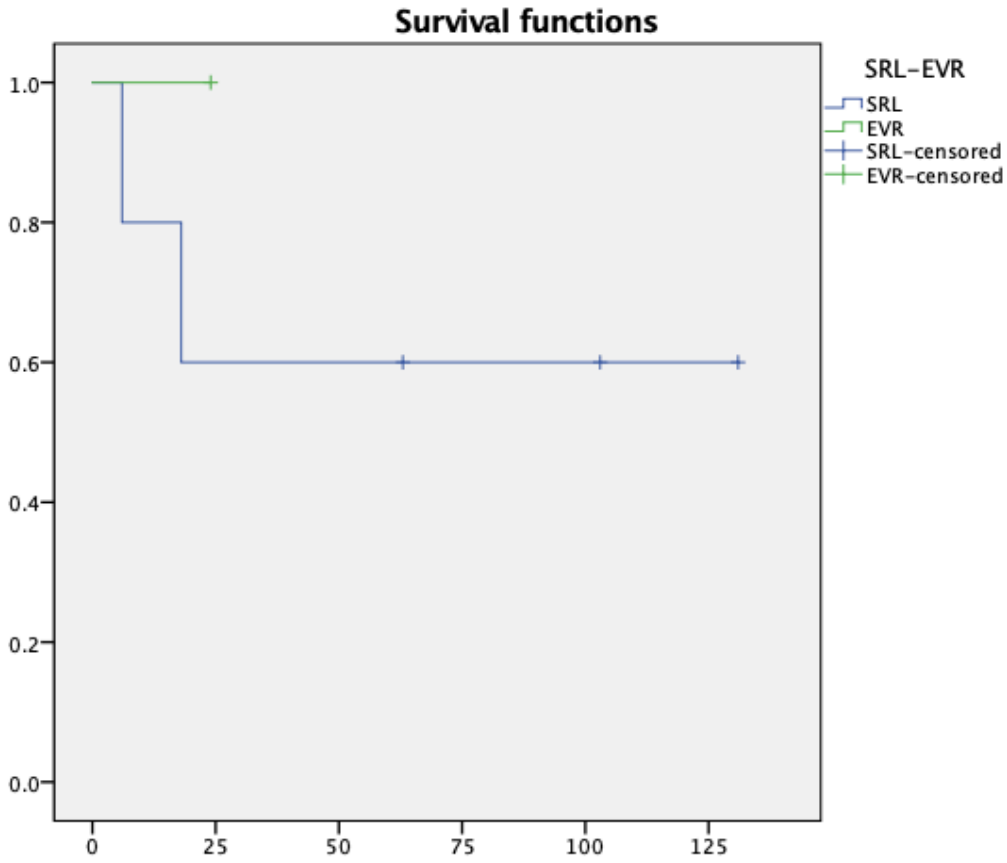


Figure 5.53. The survival rate of mTORi used in patients with digestive system cancer. (unit: month) (p=0.502)

There are 6 patients with mTORi therapy, and only 1 patient use Everolimus, so difference cannot be analyzed.

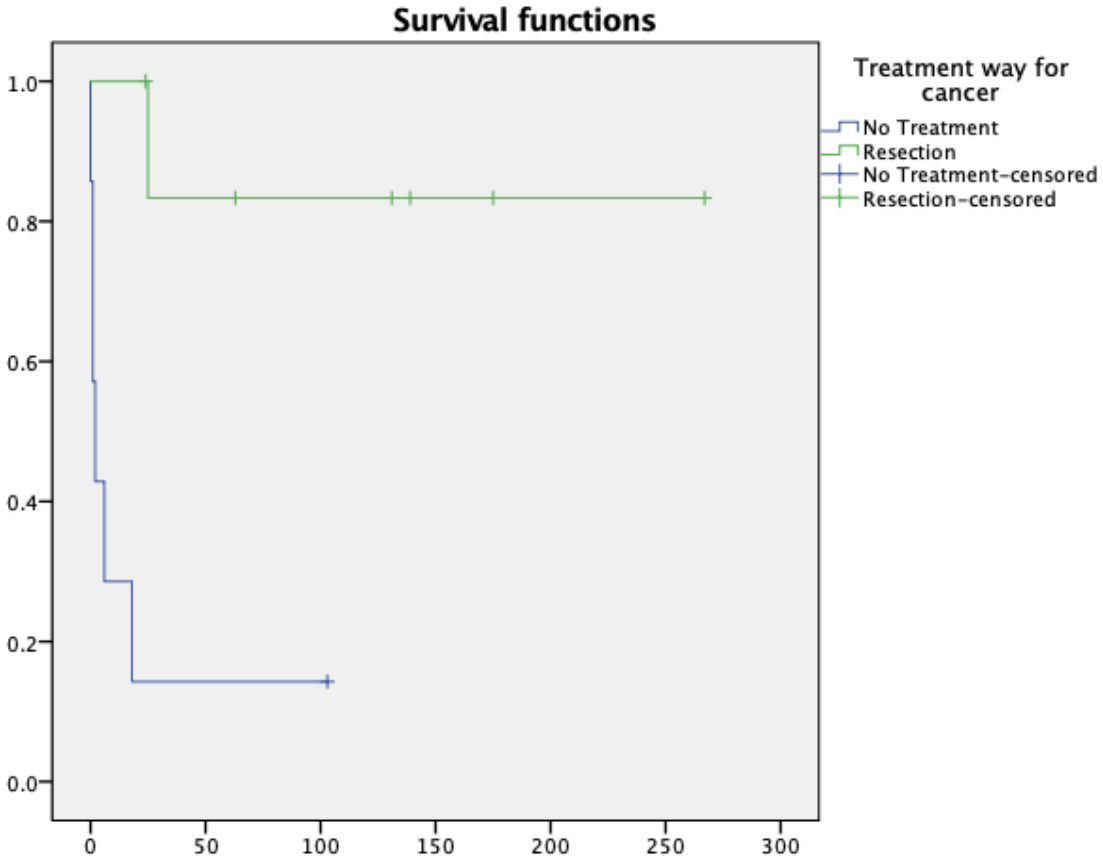


Figure. 5.54. The survival rate of treatment way in patients with digestive cancer. (unit: month) (p=0.003)

With the figure 5.54. we can see the survival of surgical resection is the best, and in these patients, there is no one with chemotherapy or radiotherapy.

Lymphoproliferative syndrome (SLPT)

Next figures shows difference in survival of liver transplant patients with SLPT depending of immunosuppressant regimen

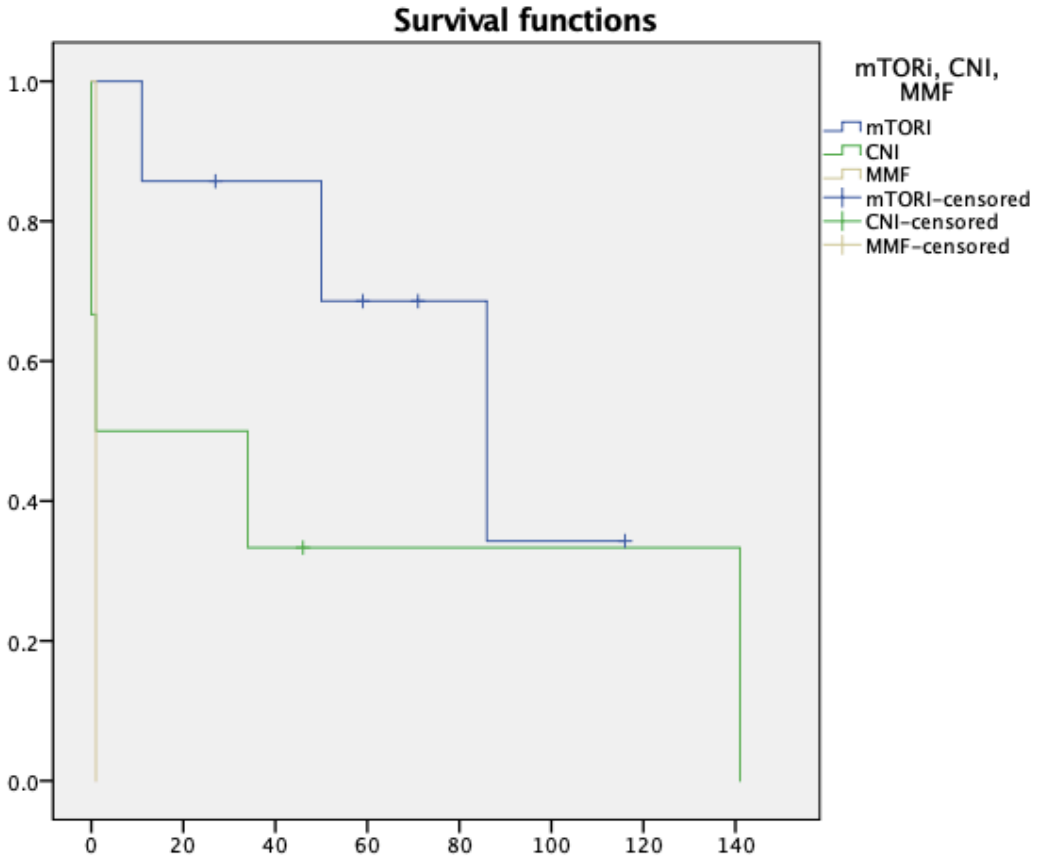


Figure 5.55. The survival rate of inhibitor used in patients with lymphoproliferative syndrome. (unit: month) (p=0.185)

The mTORi is the best of these 3 inhibitor, although the largest survival time (more than 140 months) is in patient with CNI. These differences are with p>0,05

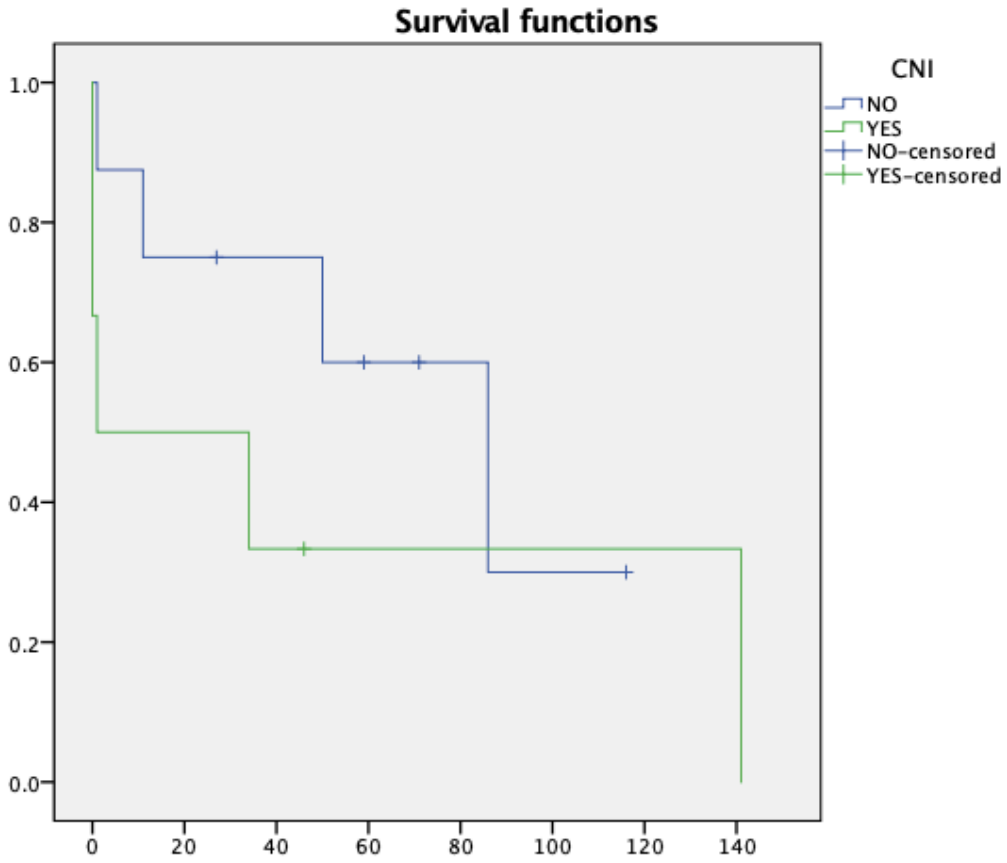


Figure 5.56. The survival rate of inhibitor used in patients with lymphoproliferative syndrome. (unit: month) (p=0.313)

The survival rate of without CNI therapy is better than with it. (p>0.05)

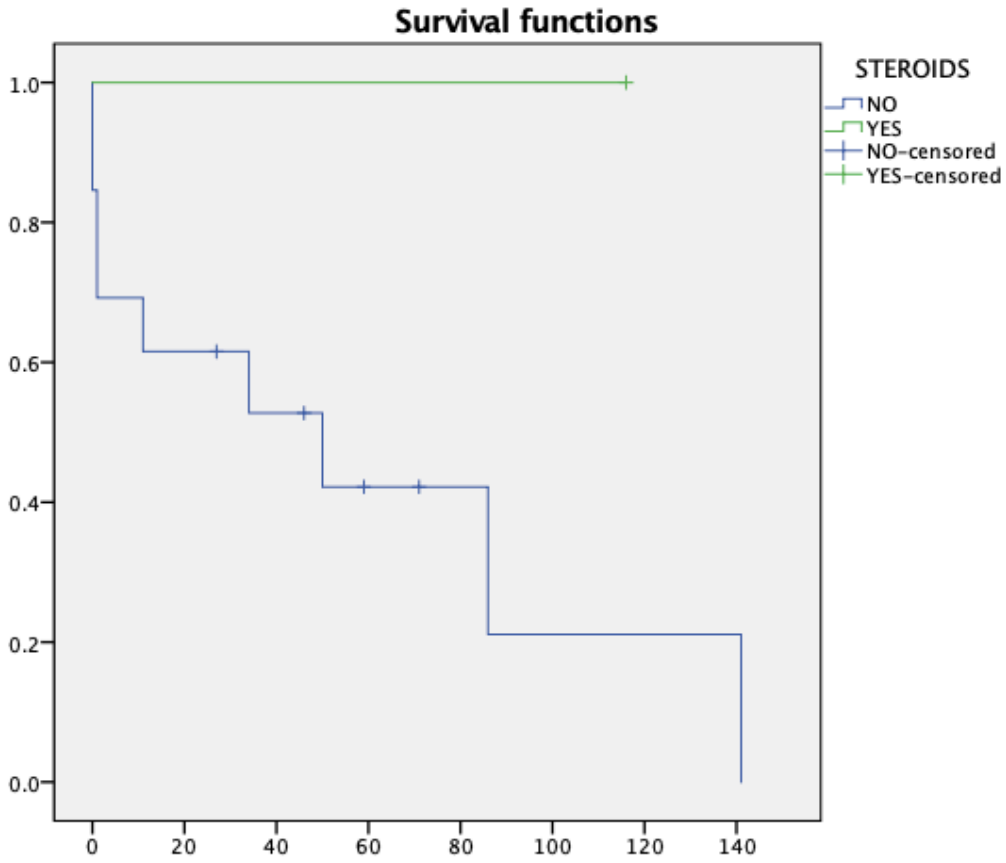


Figure 5.57. The survival rate of steroid used in patients with lymphoproliferative syndrome. (unit: month) (p=0.254)

There is 1 patient with steroid therapy, so this point cannot be analyzed.

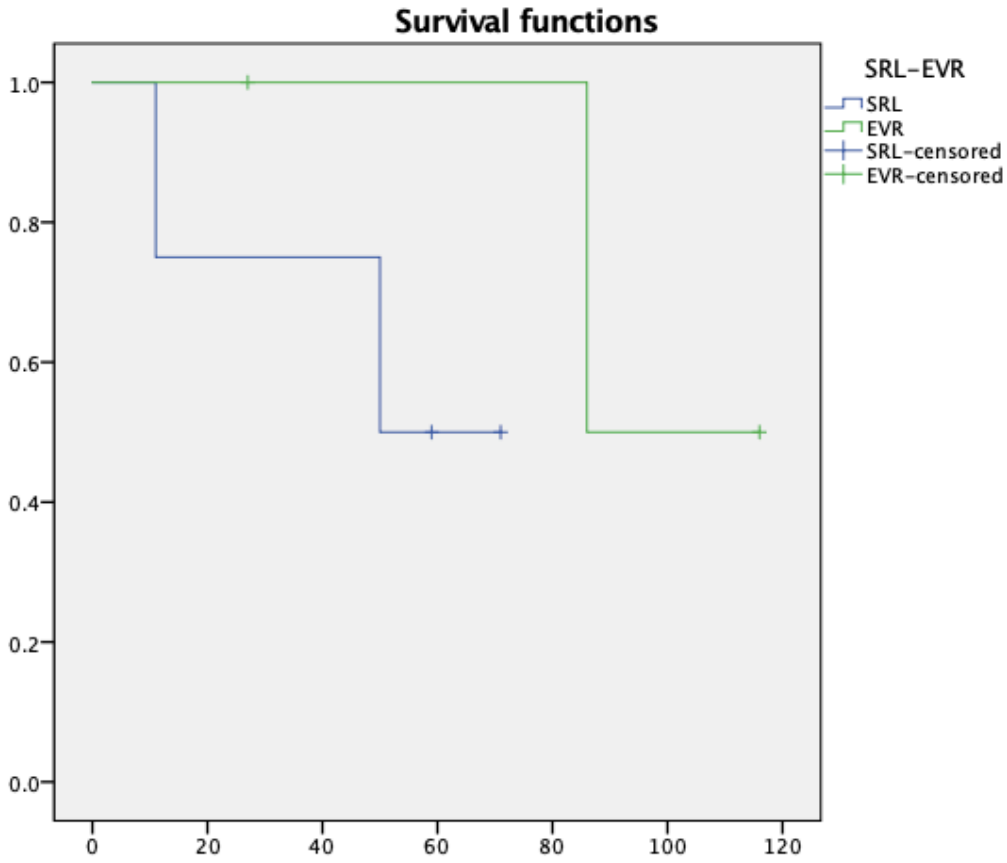


Figure 5.58. The survival rate of mTORi used in patients with lymphoproliferative syndrome. (unit: month) (p=0.234)

There are 7 patients use the mTORi and the efficacy of Everolimus in survival sems to be better than Sirolimus, although *p*>0,05 because of the few patients analyzed

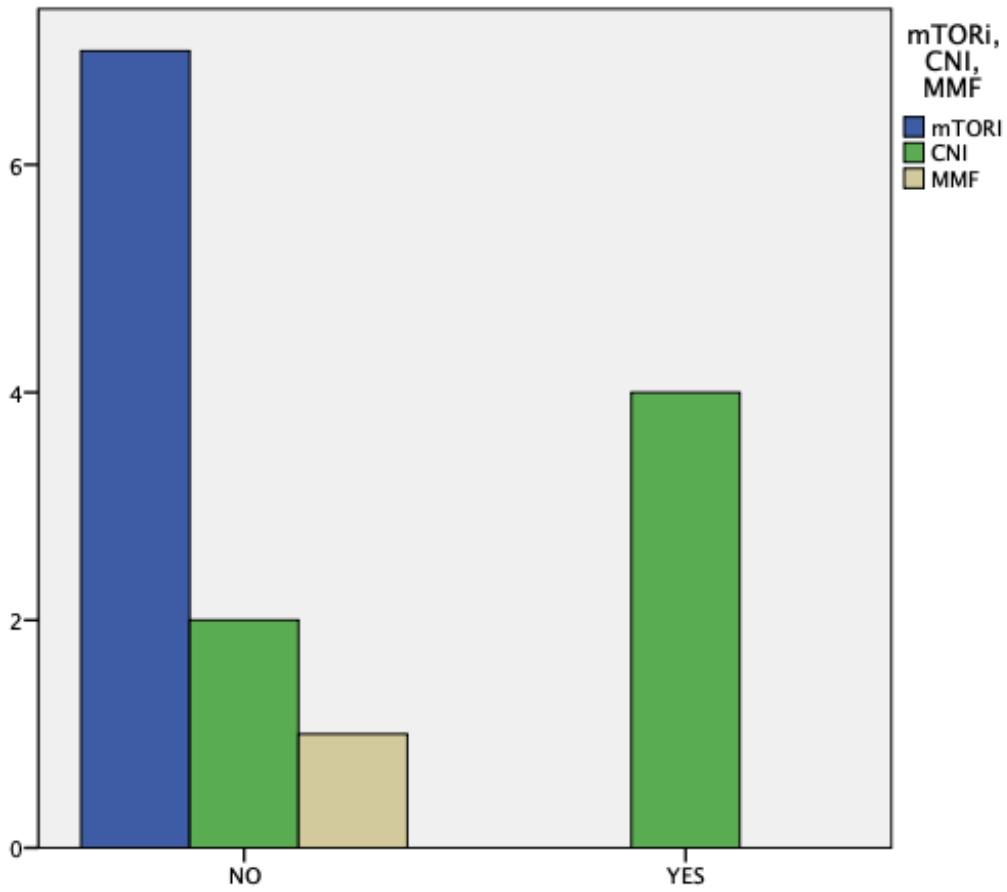


Figure 5.59. The distribution of patient used immunosuppressant with or without tumor recurrence

There are 4 patients with tumor recurrence, and all the 4 patients with CNI regimen. (p=0.024)

Breast cancer

Next figures shows difference in survival of liver transplant women with breast cancer, depending of immunosuppressant regimen

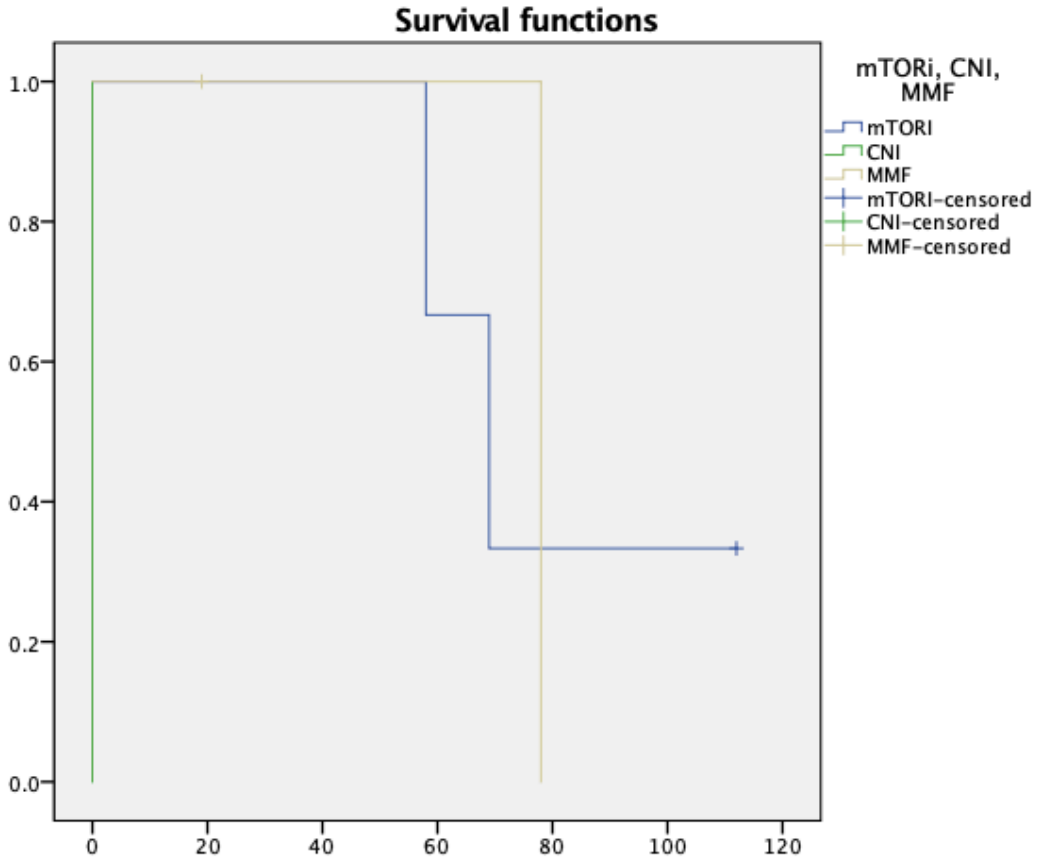


Figure 5.60. The survival rate of inhibitor used in patients with breast cancer. (unit: month) (p=0.082)

There are 6 patients with breast cancer, 1 patient with CNI therapy, 3 patients with MMF therapy and 3 patients with mTORi therapy. In the survival test, the MMF and mTORi are similar, but MMF is better. The 6 patients without steroid therapy and all the 3 patients with mTORi were use Sirolimus.

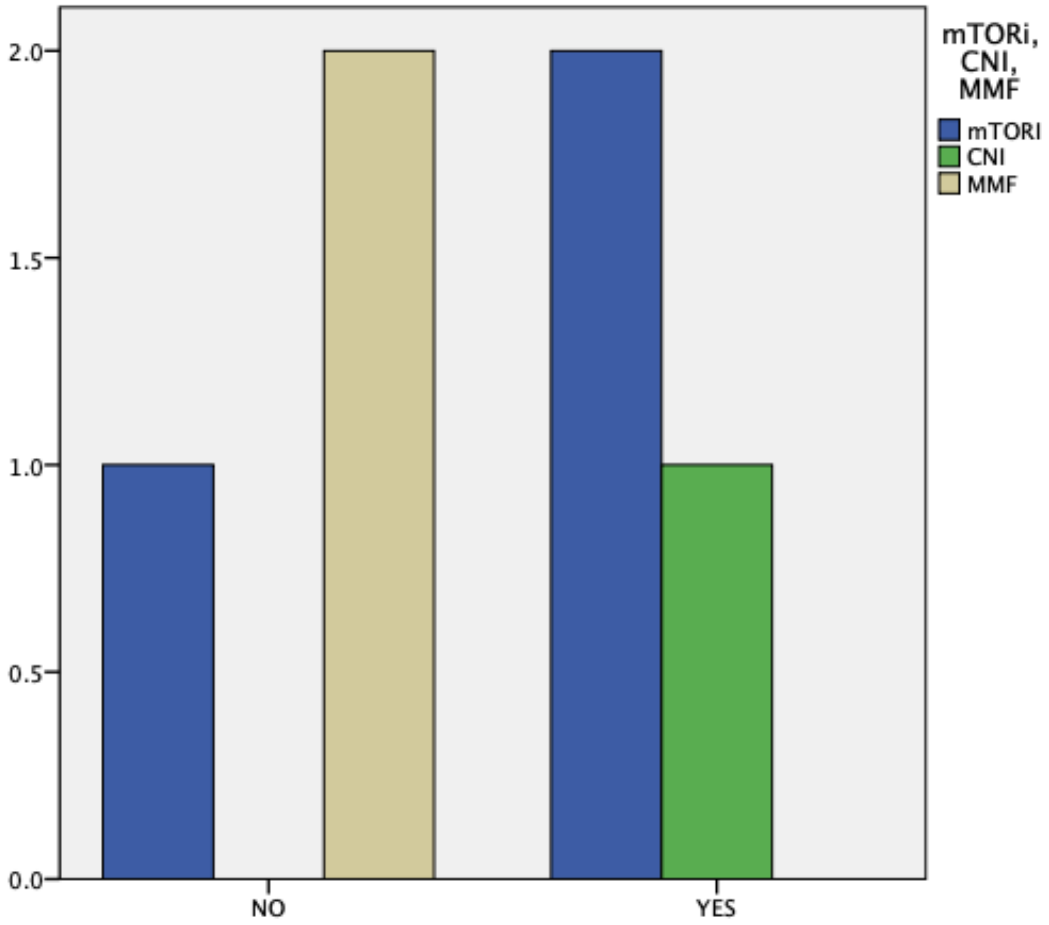


Figure 5.61. The distribution of patient used immunosuppressant with or without tumor recurrence

There are 3 patient with tumor recurrence, 2 patients with mTORi regimen and other 1 patient with CNI regimen. (p=0.189)

Urinary system cancer

Next figures shows difference in survival of liver transplant patients with urinary system cancer (kidney cancer, urothelial cancer, prostatic cancer), depending of immunosuppressant regimen

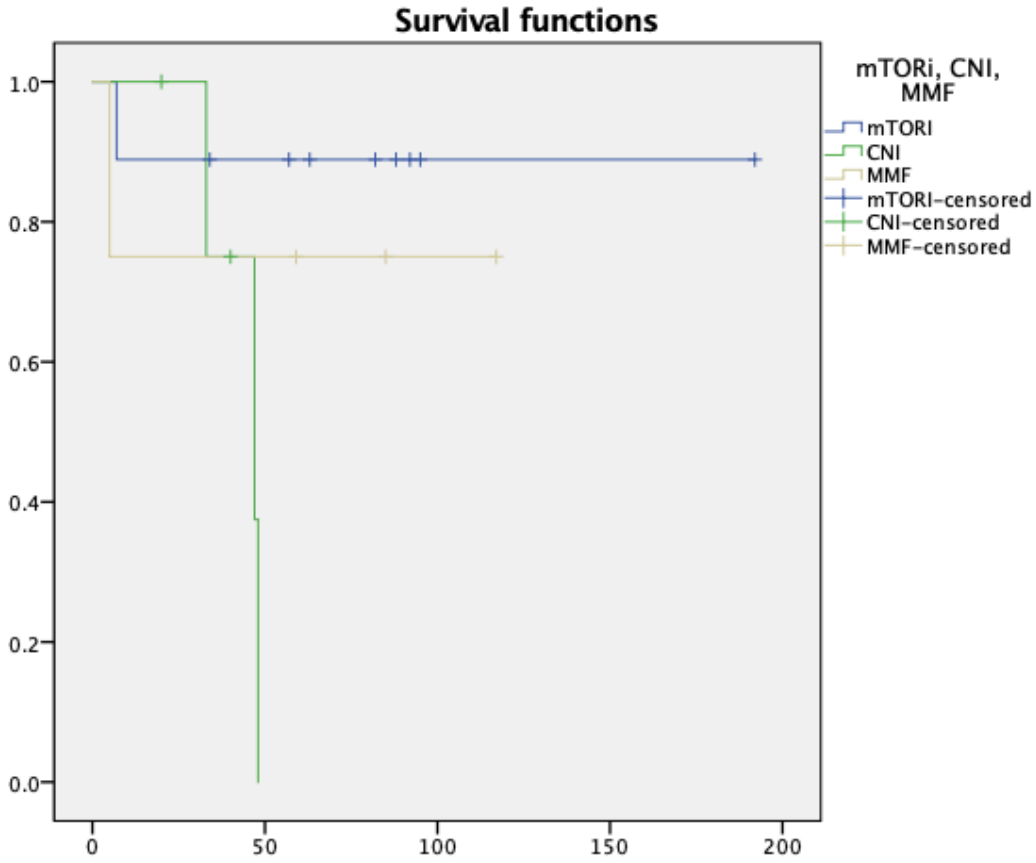


Figure 5.62. The survival rate of inhibitor used in patients with Urinary system cancer cancer. (unit: month) (p=0.100)

In the survival test, the effective of mTORi (n=9) is the best, MMF (n=4) is between mTORi and CNI (n=5). This is only a clinical observation, with *p* near to 0.05.

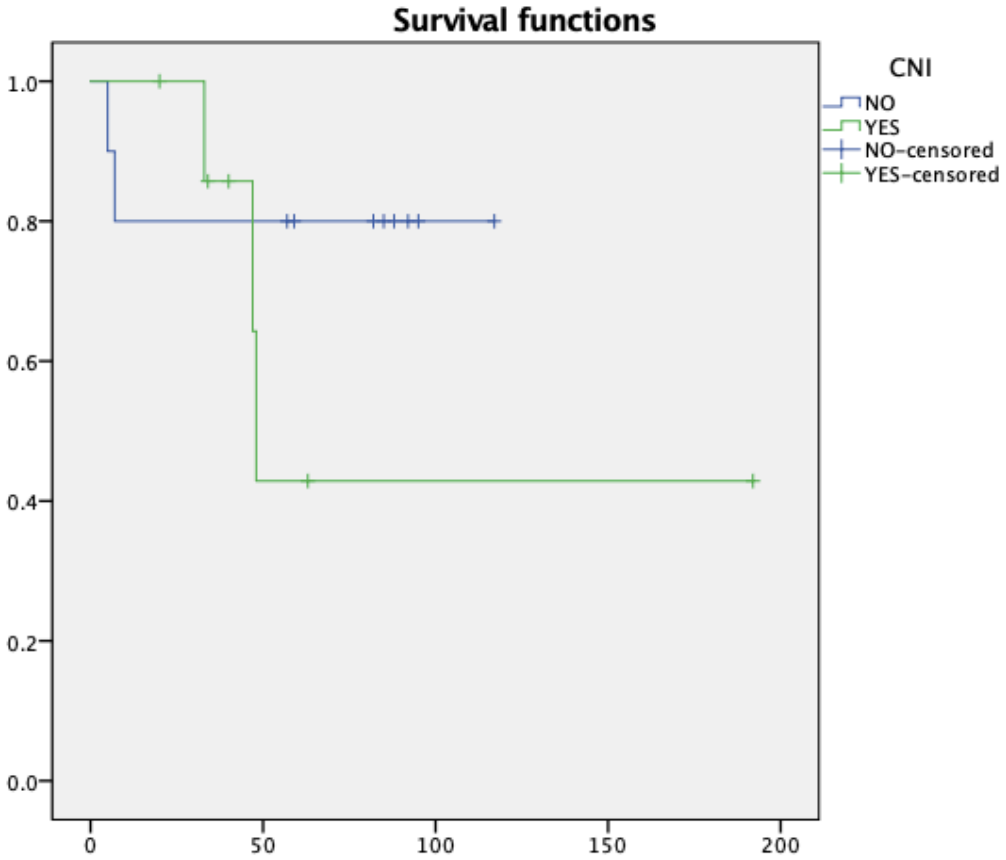


Figure 5.63. The survival rate of CNI used in patients with Urinary system cancer cancer. (unit: month) (p=0.348)

In the short-term (50 months), with CNI therapy is better, and after 50 months, without CNI therapy is better and the different is almost 40%. (p>0.05)

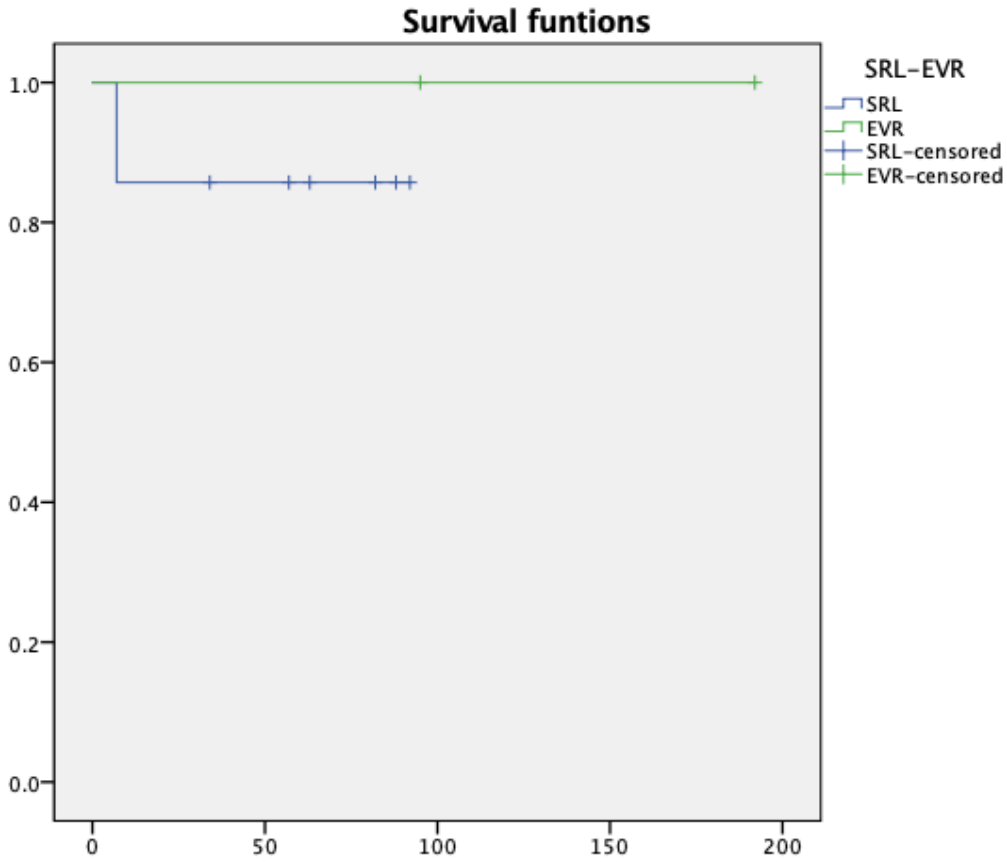


Figure 5.64. The survival rate of mTORi used in patients with Urinary system cancer cancer. (unit: month) (p=0.593)

There are 2 patients with Everolimus and 7 patients with Sirolimus. In this survival test, the survival rate of patients with Everolimus is better than Sirolimus. (p>0.05)

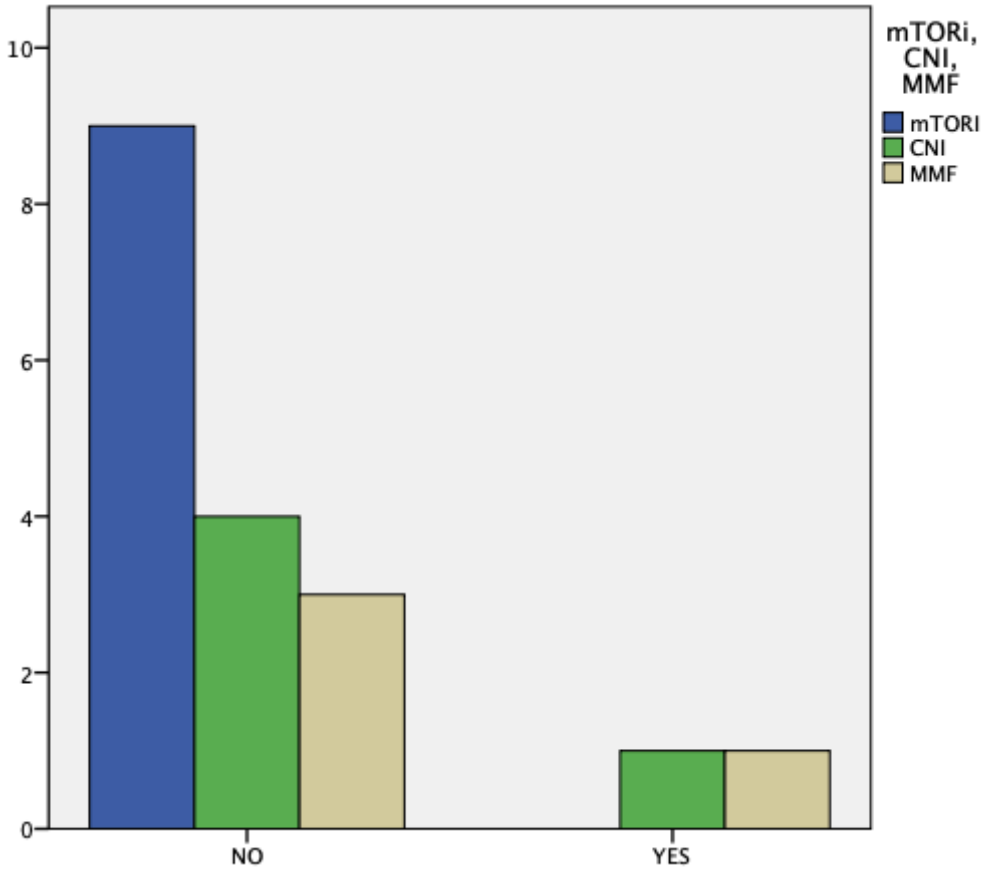


Figure 5.65. The distribution of patient used immunosuppressant with or without tumor recurrence.

There are 2 patients with tumor recurrence and 16 patients without tumor recurrence. In these 2 patient, 1 with CNI and other with MMF. (p=0.316)

Hepatobiliary cancer

Next figures shows difference in survival of liver transplant patients with HPB cancer, depending of inmunosupressant regimen

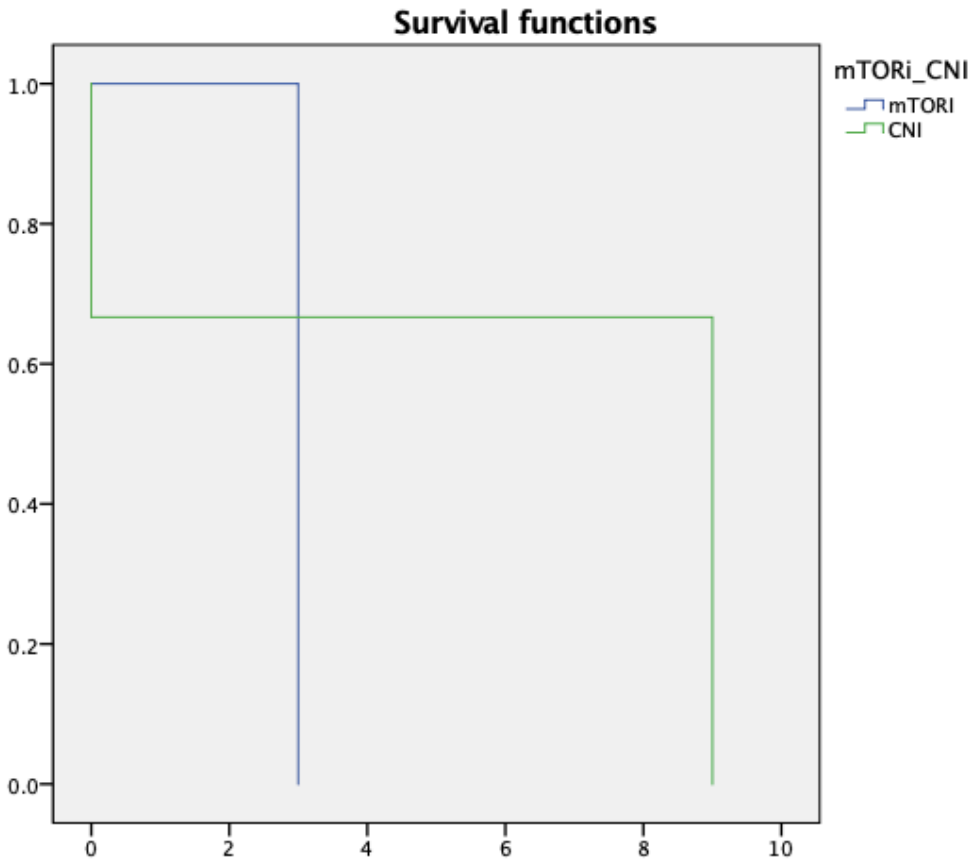


Figure 5.66. The survival rate of inhibitor used in patients with Hepatobiliary cancer (without HCC). (unit: month) (p=0.515)

There are 4 patients with hepatobiliary cancer, due to the HCC is a separate chapter. And in these patients, 3 patients with CNI and 1 with mTORi (Sirolimus).

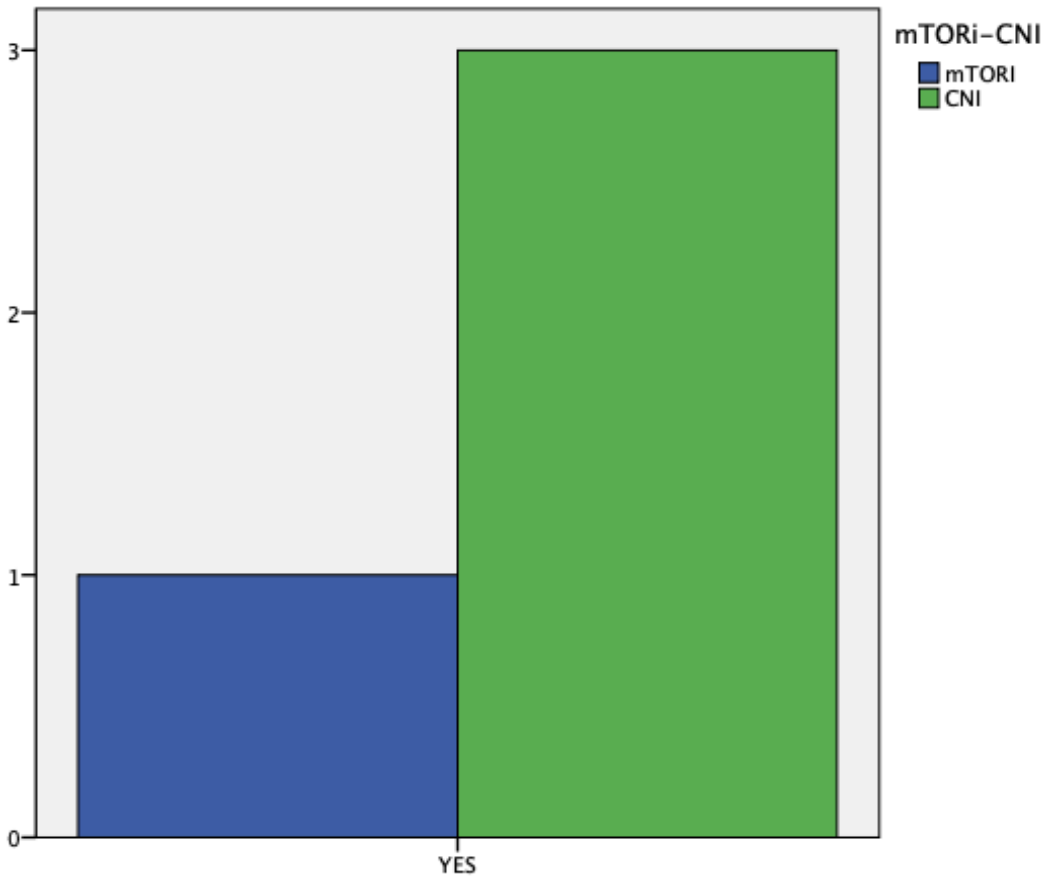


Figure 5.67. The distribution of patient used immunosuppressant with or without tumor recurrence

All the 4 patients with hepatobiliary cancer have been recurrent tumor. 1 with mTORi regimen and others 3 patients with CNI regimen.

Multivariant analysis of influence of mTORi in NN

Effect	Odds ratio	95% Confidence interval	Valor (p)
Skin cancer	1.8	1.6 – 2.5	0.19
ORL cancer	2.5	1.2 – 2.4	0.05
Lymphoproliferative syndrome	4.8	2.8 – 7.2	0.03
Digestive cancer	1.3	1.2 – 2.1	0.23
Urinary cancer	4.6	3.2 – 6.8	0.04
Breast cancer	0.7	0.6 – 0.9	0.36

Tab. 5.14.

6. DISCUSSION

6. Discussion

There are many study describing safety of mTORi as a immunosuppressant therapy after liver transplantation. Although the adverse effective of mTORi, including the distal edema, dermatological adverse effective, oral mucositis, diarrhea, abdominal pain etc., these drugs can be used to avoid rejection in these patients.

mTORi reduce the incidence of the complications after operation liver transplantation including nephrotoxicity, neurotoxicity, hypertension and *de novo* DM.

As we known, the de novo neoplasia is a important factor risk to affect the survival time in patient after liver transplantation because of immunotherapy drugs. And the patients after liver transplantation need to keep intake the immunosuppressive agent. So, how to control the dose or choose the type of immunosuppressive agent for find out the balance to make the patient have a better living after LT is the final objective that we want to reach.

The treatment way is a important factor to survival. The most important therapy in survival of patients with cancer is resection or chemotherapy/radiotherapy. In liver transplant patients, immunosuppressant regiment could help or be worst for the curation of the tumor. In this study, most of patients is resection, the second is chemotherapy or radiotherapy and the worst way is no treatment. The survival time and rate of resection is very higher than others two. Why there are some patients without the treatment resection? It's depend the situation of patient, the stage of cancer and the overall status. Some patients with the cancer in end-stage when founded or not meet the criteria of resection.

In liver transplant patients because of HCC or in those patients with “de novo” neoplasia, immunosuppressant treatment could be harmful for the treatment of the cancer. CNI and MMF have demonstrated reduce immunity in the patient and let the tumor grows. However, an anti-neoplastic activity has been demonstrated for everolimus with regard to various solid tumors (kidney cancer, lung cancer, …), and a potential role in HCC and cholangiocarcinoma are being increasingly reportrd[113].

In this study, we make an analysis of diverse factors that could have effect on the survival of patients with neoplasia de novo or those transplanted because an HCC, as smoke, alcohol, histological characteristic, treatment method and immunosuppressant scheme.

This study analyzes the patients after liver transplantation with inhibitor CNI, mTORi and MMF.

6.1. Hepatocellular carcinoma (HCC)

6.1.1. Is there influence in survival about smoking and alcohol?

We have observed that tobacco and alcohol reduce the survival time. In the short-term, the effective of smoke is not clear, even the survival time of smoking patient is higher in the first 50 months. But in the long-term, the survival rate of patient without smoke is better. For intaking alcohol, whatever short-term or long-term, no intake alcohol is much better than alcoholic intake. More than 60% patients without alcohol intaking and theirs’ survival time can achieve to 300 months, lees than 20% patients with alcohol intaking can reach to 200 months. So, smoking and alcohol consumption reduce the survival time. There are some studies published also describe the influence of smoking and alcohol for HCC, that smoking and alcohol consumption increase the

risk of HCC occur[114,115]. And there is a study indicate that smoking and alcohol consumption after liver transplantation can reduce the survival time[116].

6.1.2. Do histological characteristics affect patient survival?

About the histological characteristic, including the vascular invasion, capsule appearance and Edmondson. We observe the related of survival time and vascular invasion, almost 80% patients without vascular invasion reach 50 months, and more than 60% patients reach 150 months. However, just 40% patients can reach 220 months. Other side, less than 50% patients with vascular invasion can reach 50 months, and the largest time of survival is 183 months, just 3 (7.3%) patients' survival are more than 150 months. The patients without capsule appearance, the survival time is much better than the patients with capsule appearance. More than 60% patients without it achieve 150 months after liver transplantation, however, the patients with it just 1 patient's (3.12%) survival time more than 150 months. Most of these patients with it, the survival time is about 50 months. Other point worth to attention, about the Edmondson. As we known, to the survival time and prognosis, the highly differentiated tumor will be the be better, and the poorly differentiated tumor is bad. But there is a unusual survival rate in our analysis that the poorly differentiated is higher than the moderately differentiated and the highly differentiated at the first few months, although back to the usual situation few months later. May in the first few months, the effective is not clearly or the patients with poorly differentiated is not enough. In several studies indicate that vascular invasion is related to poor prognosis and HCC with vascular invasion have a higher recurrence rate[117]. Same to the vascular invasion, the capsule appearance and Edmondson are the factor risk to reduce the survival time and increase the recurrence rate[117,118].

6.1.3. Survival in the patients with recurrence of HCC

In this study, there are 17% patients with recurrence of HCC. Of these patients, 82% patients are HCC without recurrence, 10% patients occur with metastasis and 7% patients occur with local recurrence without metastasis. The survival rate of patients without recurrence of HCC is higher than the patients with recurrence of HCC. And 40% of these can achieve 300 months. Due to mentioned above, smoking, alcohol consumption and the histological characteristics have influence in recurrence of HCC. In spite of this, HCC's recurrence is a complicated situation with many factors and causes. Also there are several articles point out that the influence of HCC's recurrence to the survival of patients after liver transplantation[119,120].

At the same time, we make the comparing of the location of HCC's recurrence. The patients without recurrence and the survival time are much better than other two. About 70% patients reach 150 months (12.5 years), more than 80% patients achieve 50 months, 40% patients' survival time more than 200 months. 60% patients with recurrent tumor in location local can reach 50 months, but the patients with metastasis just 30% of these can reach 50 months. There is a article describe about the location of HCC's recurrence and the survival time. Same to our result, the survival time of HCC recurrence is better than HCC recurrence with metastasis[119].

6.1.4. Is there influence of survival about Immunosuppressant scheme?

According to data from the Spanish Hepatic Transplant Registry (RETH), Hepatocellular Carcinoma is one of the main indications for transplantation in Spain, reaching more than 20% of annual transplants. Likewise, and being the only organ that transplants a type of cancer, the immunosuppressive management in these patients should be peculiar and especially individualized.

Based on retrospective studies, a direct correlation was found between high levels of calcineurin inhibitors (CNI) in the first months post-transplant and the risk of tumor recurrence in transplant patients according to the Milan criteria. Therefore, it is advisable to avoid overdosing of the CNI, these levels oscillating in: Tacrolimus <10 ng / ml and Cyclosporine <300 ng / ml.

In relation to Hepatocellular carcinoma (HCC), two possible immunosuppression scenarios are proposed:

A.- Role of mTORi in the prevention of HCC relapse:

Several retrospective studies and systematic reviews show that mTORi reduce the risk of tumor recurrence in patients with HCC. However, the scientific evidence is very low.

The use of mTOR inhibitors in high-risk patients has been generalized in clinical practice (Alpha-Fetoprotein> 200ng / ml, HCC exceeding the Milan Criteria in the explant piece, vascular invasion, poorly differentiated tumor) . In spite of this, it has not been demonstrated that this clinical practice supposes a benefit for the patient.

There is not enough scientific evidence to recommend the widespread use of mTOR inhibitors to reduce the risk of recurrence after liver transplantation.

B.- Role of mTOR inhibitors in the treatment of CHC relapse:

There is no evidence that the use of mTORi improves the prognosis of patients with HCC relapse.

There are small series in which the combination of mTORi and sorafenib has been used safely. There are no clinical trials that demonstrate a survival benefit.

To the patients after liver transplantation, the effective of mTORi is better than CNI. More than 80% patients' survival time beyond 50 months who used mTORi. The patients with CNI, about 70%, even less than 70% achieve 50 months (p=0.051). 60% patients with mTORi achieve 200 months, less than 40% patients with CNI reach 200 months and rare patients' survival time reach 250 months. The MMF's effective is the best, but only 3 patients with this regimen, so it is only a clinical observation without statistical confirmation. It is only a clinical observation without statistical confirmation. In the 86 patients use mTORi, there are 37 patients use Sirolimus and 49 patients use Everolimus. Due to the test, the effect of Everolimus is better than Sirolimus. The most difference is in the point 100 months, about 40%, but continue to 125 months, the difference is about 20%.(p=0.018)

As mentioned in introduction that CNI as the main immunosuppressant for patients after liver transplantation in past years. Considering of the toxicities including: nephrotoxicity, neurotoxicity, hypertension, *de novo* DM and these adverse effecting can increase the incidence of *de novo* neoplasia. The new immunosuppressant had been developed: the mammalian target of rapamycin inhibitors (m-TORi). mTORi has a different mechanism to CNI, it reduce the incidence of *de novo* neoplasia and HCC recurrence[121], and increase the survival after liver transplantation. In our study, the survival time of patients used mTORi are higher than CNI. CNIs have been documented to have adverse impact on cancer in in vitro and in vivo animal studies and to have dose related adverse impact on HCC recurrence and survival in clinical series[121].

Everolimus and Sirolimus are the two immunosuppressant belong to mTORi with similar mechanism, although they have different pharmacokinetic, pharmacodynamic and toxicodynamic properties. We make the comparing of effective of these two inhibitor and get the result that Everolimus' effective is better than Sirolimus, but there is no article published compared the effective of survival in these two inhibitor, although

they describe the difference from other side such as renal function, dyslipidemia, hematology, edema and infections et. al.[121].

We also make the survival test of therapy with CNI or no and about Steroid. The result is that without CNI's survival is better, same to Steroid. As the mentioned in introduction that Steroid is the choose in the early-phase liver transplantation and with many adverse effecting. It has been replaced by others drugs like CNI and mTORi.

6.2. mTORi in *"de novo"* neoplasia after LT

6.2.1. Do smoking and alcohol have influence on survival?

Like the result of HCC, the survival of patients without smoke and alcohol is higher than with it. Smoke is the cause can affect many cancer such as lung cancer, otorhinolaryngologic cancer, and is the factor risk for cardiovascular disease. Same to smoke, alcohol consumption also is the factor risk of de novo neoplasia for patients after OLT and can increase the incidence of de novo neoplasia. There are several articles has been described that the alcohol consumption ant tobacco are well-known to be associated with these tumor because the carcinogenic or co-carcinogenic effects of smoking and drinking might be enhanced by the post-LT immunosuppressive therapy. And the active smoking was revealed as one of the major risk cofactors, independent of alcoholic relapse, of long-term morbidity and mortality in transplant recipients, either from cardiovascular complications or from de novo neoplasms. In general, the drinker usually is a smoker, because we now acknowledge that tobacco is a risk factor for alcohol abuse. So, smoke and alcohol intaking increase the incidence occurring of de novo neoplasia. There are several studies indicate in the last 10 years, alcohol liver disease is the LT indication that has seen the greatest increase in

prevalence as well as in post-LT survival rate compared with other causes of liver disease[122,123].

6.2.2. Is there influence in survival about treatment of the tumor?

In our study, the resection is the best way to treatment tumor in *de novo* neoplasia, with a 45% of survival in 20 years (p=0). Chemotherapy or Radiotherapy and no treatment is similar, with no survival at 10 years, but Chemotherapy or Radiotherapy is little better than without treatment in the short-term. Due to the conditions of treatment, when the tumor has been advanced to end-stage or cannot be resection, medico should choose the chemotherapy or radiotherapy to reduce the stage of tumor till reach the criterion of resection. Or if the tumor is advanced or with metastasis distal, it may not get the result that hoped. Considering the socially side, doctor and the patient may choose no treatment. But there is not an article to discuss about this relation to LT patients.

6.2.3. The influence in survival of cancer staging

We make the analysis of the stage of cancer and get the result: about the stage T, the survival time of stage 1 of T is best. As we know, the stage 4 is severe, so the worst is stage 4, the stage 2 and stage 3 are similar but the stage 2 is better than stage 3; about the stage N, the stage 0 with the best survival time and others are similar and bad; about the stage M, the survival time without metastasis distal is more better than with it. It's well-known the effective about stage of cancer that N is describe the size of tumor, N about lymph node invasion and M is describe the metastasis distal. Whatever the novo neoplasia in patient after LT or general population, a tumor with metastasis

means the cancer has been entre end-stage or advanced and there are few treatment for this situation. Certainly the N and T stage mean the developing of the cancer.

6.2.4. The influence of immunosuppressant treatment in neoplasia de novo.

mTORi in “de novo” neoplasia after LT

“*De novo*” neoplasms are an important cause of morbidity and mortality after liver transplantation. The recipients of transplants of solid organs have a greater risk of neoplasia than the general population. This is an important cause of mortality.

In relation to de novo neoplasms, there are two possible scenarios in which to assess the role of immunosuppression:1) the possible changes in immunosuppression that can be carried out to try to prevent the development of de novo neoplasms and 2) the changes in the immunosuppression in patients who have developed neoplasms.

The first action to try to avoid the development of de novo neoplasms should be to act on the risk factors of neoplasia such as smoking, alcohol or solar radiation, which are important risk factors both in the general population and in transplant patients (papers on tobacco, alcohol, solar radiation). Acting on these factors can reduce the risk of neoplasia, as shown by the fact that transplant patients who quit smoking have a lower risk of developing neoplasia (LT over tobacco).

Several studies have evaluated the role of calcineurin inhibitors (CNI). Some studies suggest that patients treated with tacrolimus develop de novo neoplasms more frequently than those treated with cyclosporine, possibly due to the greater

immunosuppressive power of tacrolimus. There are also studies that attribute a greater oncogenic capacity to cyclosporine. In the absence of evidence, one or the other CNI cannot be recommended.

The oncogenic role of antilymphocyte globulins (OKT3 and ATG) has been known for years. They favor the development of lymphomas and their use is associated with an increased risk of developing neoplasms in general (transplantation). Therefore, it is recommended to avoid its use. This recommendation does not affect antibodies against CD25.

The inhibitors of mTOR (mammalian target of rapamycin) (mTORi) sirolimus and everolimus have antiproliferative capacity and are used as chemotherapeutic agents in cancer patients. For this reason, there has been much hope that immunosuppression using these drugs will reduce the risk of neoplasia. Patients receiving immunosuppression with mTORi have less risk of developing non-melanoma cutaneous neoplasms, both in liver transplantation and kidney transplantation, but this does not justify their use as immunosuppressants in all patients, since this type of injuries very rarely is fatal. The effect of these drugs on the development of non-cutaneous neoplasms is much less clear. In clinical trials with several years of follow-up and with important numbers of patients, no differences have been found in the incidence of non-cutaneous neoplasms between patients treated with mTORi and in patients who maintain treatment with CNI.

It is not clear that the intensity of the immunosuppressive treatment is associated with a higher or lower prevalence of neoplasia. Some studies suggest that a more potent immunosuppressive treatment may predispose to an increased risk of neoplasia. On the other hand, some side effects of immunosuppression are dose-dependent, such as renal toxicity, diabetes, dyslipidemia, hypertension. Therefore, the recommendation is to avoid excessive immunosuppression.

In some cases in which a post-transplant neoplasm has developed, changes in immunosuppression may be considered with a potential effect on subsequent evolution. In patients with Kaposi's sarcoma, substitution of ICN by mTORi favorably influences the disease. On the other hand, the reduction of immunosuppressive potency in patients with post-transplant lymphoma was associated with better evolution.

In patients with cutaneous non-melanoma tumors, substitution of CNI by mTORi reduces the risk of developing a second neoplasm. However, this change in immunosuppression is not considered justified since these tumors do not pose a vital risk to the patient.

Although the weakness of the scientific evidence does not allow for a recommendation, in clinical practice it is common to substitute CNI for patients who have had a non-cutaneous neoplasia. When this strategy is used, possible interference with antineoplastic treatment should be taken into account, since mTORi can increase the medullary aplasia caused by chemotherapy. On the other hand, since they inhibit scarring they can lead to complications in case of surgical treatment.

Considering there are no statistical significance in some cancer because of the little number of patients, we have made the Kaplan-Meier test with all patients with *de novo* neoplasia. The test has shown that mTORi and MMF are better than CNI. In the short-term, MMF is similar with CNI, and mTORi is better than others two. In the middle-term, the MMF is the best in these 3 inhinitor, however, later than 80 months, the effect of MMF is worse than mTORi. Obviously, CNI is the worst in these 3. Compare in two inhibitor of mTORi. Due to the Kaplan-Meier test (with the p=0.042), the effect of Everolimus is better than Sirolimus. And theirs different is about 10%. There are some articles indicate de novo neoplasia is a very significant cause of mortality, particularly for long-term survivors, and minimization of long-term immunosuppression should be aimed at reducing the incidence of de novo neoplasia.

There is growing evidence that the incidence of neoplastic disease is inferior in patients with gradual reduction of CNI with the introduction of mTOR inhibitors, vs those subjects treated with standard-dose CNI.

There are few articles talking about mTORi treatment in liver transplant patients with neoplasia de novo[124].

mTORi in digestive system cancer

We have associated in this cancer all colorectal, gastric and esophagus tumors (n=14). In test survival (Kaplan-Meier), the mTORi's effect is the best of the 3 drugs we study (CNI, mTORi, MMF), and the survival without CNI is better than with CNI, although p>0,05 probably associated to the litte sample size.

Compared the patients use Everolimus and Sirolimus, there is 1 patient use Everolimus, and cannot present the different clearly. There are no articles talking about mTORi treatment in liver transplant patients with digestive system tumors.

mTORi in Skin cancer

For the skin cancer, we have not found differences in survival depending of type of immunosuppressant therapy. In the first 50 months, the MMF is better than others two (n=55, survival 100% vs 88% and 80%, p=0.954); between the 75 months and 120 months, the CNI is better than others(82% vs 77% and 70%); and after 120 months, the mTORi shown better effect. In the short-term (about 50 months). Also, there is no differences between everolimus and sirolimus (n=22, p=0.866, survival 88% vs 78%). About CNI, with and without it are not obvious, after 150 months, without CNI's rate is higher than other. Without steroid therapy is much better than patient with the steroid therapy. Considering the stage of skin cancer and the early diagnosis, the treatment

method always be resection. The survival time is better than the non-skin cancer. And many articles indicate they get the same results[125].

mTORi in Otorhinolaryngologic cancer

We have made the survival test to check the inhibitor's efficacy in ORL cancer and get the result. The resection is the best treatment method for liver transplant patients with Otorhinolaryngologic cancer (n=31, p=0.03). MMF treatment is better than mTORi and CNI in prolong the survival of these patients ($p<0.05$). Also, mTORi is better than CNI. Without CNI's therapy is better than CNI therapy (p=0.018). The effect of Everolimus seems to be better than Sirolimus (n=16, p=0.159). Because there is no patients with Steroid therapy, it cannot present the difference of with or without it. And the results as I expected that Everolimus with the best effective in all inhibitors in our study. May it related the Everolimus is the second generation mTORi. There is no articles talking about this fact in liver transplant patients with ORL cancer.

mTORi in Lung cancer

In our study, the most of patients (14 patients) with novo lung cancer is in advanced stage, only 5 patients to meet the conditions of resection. Although the number of patients with resection is less than others, for the survival time, resection is the best treatment method to solution cancer. About inhibitors, mTORi is better than others 2 drugs CNI and MMF(p=0.067), CNI and MMF are not different clearly although CNI is a little better, and the survival time is largest that almost reach 50 months. In the first 15 months, without CNI therapy is better than CNI therapy. So, with and without CNI therapy are not different clearly. Sirolimus has no a good effective when we compared Sirolimus and Everolimus. As we mentioned in result chapter, in the survival time, everolimus therapy is more than 2 times than patients with sirolimus. Due to the developing of lung cancer is fast, the survival is less than 50 months.

mTORi in lymphoproliferative syndrome

The mTORi is the best of these 3 inhibitor (p=0.185), although the largest survival time (more than 140 months) is by patient with CNI. And only 1 patient use MMF. The survival rate of without CNI therapy is better than with it. There is only one patient with steroid therapy, so we cannot conclude anything. There are 7 patients use the mTORi and the efficacy of Everolimus seems to be better than Sirolimus.

In the last 5 patients with SLPT we have treated these patients with mTORi and rituximab, with great results[124].

There are no articles talking about mTORi treatment in liver transplant patients with SLPT

mTORi in Breast cancer

There are 6 patients with breast cancer, 1 patient with CNI therapy, 3 patients with MMF therapy and 3 patients with mTORi therapy. In the survival test, the MMF and mTORi are similar, but MMF is better. The 6 patients without steroid therapy and all the 3 patients with mTORi were treated with Sirolimus. In our study, all patients with novo breast cancer were resection. Due to the limitation of data and result, the analysis dose not statistical significance.

mTORi in Urinary system cancer

In the survival test, the survival is better with mTORi (n=9) is the best, MMF (n=4) is between mTORi and CNI (n=5). In the short-term (50 months), with CNI therapy is better, and after 50 months, without CNI therapy is better and the different is almost 40%. There are 2 patients with Everolimus and 7 patients with Sirolimus. In this survival

test, the survival rate of patients with Everolimus is better than Sirolimus. Like the result present that survival of CNI reach 50 months and the mTORi reach almost 200 months. Everolimus seems to be also the best treatment for these patients.

mTORi in Hepatobiliary cancer

Excepting HCC, studied apart, we analyze the others hepatobiliary cancers such as pancreatic cancer, hepatic sarcoma and cholangiocarcinoma. There are 4 patients with hepatobiliary cancer. And in these patients, 3 patients with CNI and 1 with mTORi (Sirolimus). In this survival test, CNI's effective is better and reach 9 months, Sirolimus reach 3 months, but the sample size is too short to get some conclusion.

7. CONCLUSION

7. Conclusions

1. Tobacco and Alcohol reduce the survival of patients after OLT in HCC and *de novo* neoplasia.

2. Vascular and capsular invasion and advanced Edmondson stage reduce the survival of patients transplanted because of HCC

3. mTORi immunosuppression, specially everolimus, increase the survival of patients after OLT in HCC compared to CNI.

4. Recurrence of HCC is not associated to treatment with CNII or mTORi

5. For the solid cancer, resection increase the survival of patients OLT with neoplasia de novo compared to no treatment or chemotherapy/radiotherapy.

6. TNM and tumoral stage are good predictors of survival in patients with neoplasia de novo.

7. mTORi immunosuppression, specially everolimus, increase the survival of patients after OLT in de novo neoplasia compared to CNI, above all in ORL cancer, lung cancer, SLPT, digestive cancer, breast cancer and urinary cancer.

8. Recurrence of neoplasia de novo is associated to treatment with CNI in ORL cancer, lung cancer, gastric cancer and kidney cancer.

9. This benifit of mTORi cannot be applied to skin cancer and HBP cancer.

10. MMF monotherapy also increase survival in ORL and breast cancer.

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