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General Introduction Part II

GENERAL INTRODUCTION

The discovery of the mammalian target of rapamycin (mTOR) pathway originates from the early 1970's when rapamycin was isolated from a soil sample obtained on Easter Island (Rapa Nui)¹. This macrolide was found to be produced by *Streptomyces hygroscopicus* and to have antifungal properties. In 1994 the mammalian target of rapamycin, a serine/threonine kinase, was identified and cloned^{2, 3}. Rapamycin selectively inhibits mTOR and could be used to elucidate the mTOR pathway. mTOR is ubiquitously present in cells throughout the body^{4, 5}; plays an important role in cell signaling and is involved in cell growth and cell proliferation in response to various stimuli, like growth factors, glucose and oxygen⁶⁻⁸.

mTOR consists of two distinct complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2)^{2, 3}. Either complex is associated with a scaffolding protein: mTORC1 with rapamycin associated protein of mTOR (Raptor) and mTORC2 with rapamycin insensitive companion of mTOR (Rictor). Activation of mTORC starts with activation of the lipid kinase phosphatidylinositol-3-kinase (PI3K)⁹, which in turn activates Akt^{3, 9}(figure 1). Once activated, Akt phosphorylates and inhibits the tuberous sclerosis complex (TSC)¹⁰ thereby activating mTOR by releasing it from the inhibitory effects of TSC¹¹. mTORC1 and mTORC2 have discrete functional roles¹²⁻¹⁴. Activation of mTORC1 results in inhibition of autophagy, cell proliferation and mRNA translation, whereas activation of mTORC2 contributes to regulation of cell polarity, of the actin cytoskeleton and, according to recent findings, to cell-cycle progression, anabolism and cell survival¹⁵.

Rapamycin acts by forming a complex with FK506 –binding protein 12 (FKBP-12) which then binds to - and inhibits mTORC1 by blocking its interaction with raptor. No direct effect of rapamycin has been shown on mTORC2^{3, 12, 13}. However, prolonged treatment with rapamycin can inhibit mTORC2 in a subset of tissues and cell lines¹⁶, possibly by sequestering the cellular pool of mTOR in a complex with rapamycin-FKBP12 thereby making mTOR unavailable for assembly into mTORC2¹⁵.

Although discovered as an antifungal agent, rapamycin, by blocking the mTOR pathway, has multiple effects. It has potent immunosuppressive properties by influencing the innate and adaptive immune system. Rapamycin inhibits T-cell proliferation by preventing transition from the G₁ to S phase. It impairs dendritic cell differentiation and function, partly by reducing (co-stimulatory) responses to allogenic stimuli and influencing antigen uptake, thereby modulating events that are associated with antigen presentation¹⁷. Furthermore, it has tolerance inducing properties, for example by increasing the frequency of regulatory T-cells¹⁸. mTOR inhibitors, first sirolimus/rapamycin (1999) and later everolimus (2001), have proven their efficacy in renal transplantation. They were first introduced in renal transplantation because of their supposed lack of nephrotoxicity, which contrasts to calcineurin inhibitors (CNI) which by their nephrotoxic side effects contribute to long-term graft failure¹⁹. mTOR inhibitors can be used to withdraw or decrease CNI dose, when calcineurin-inhibitor associated nephropathy is suspected, although

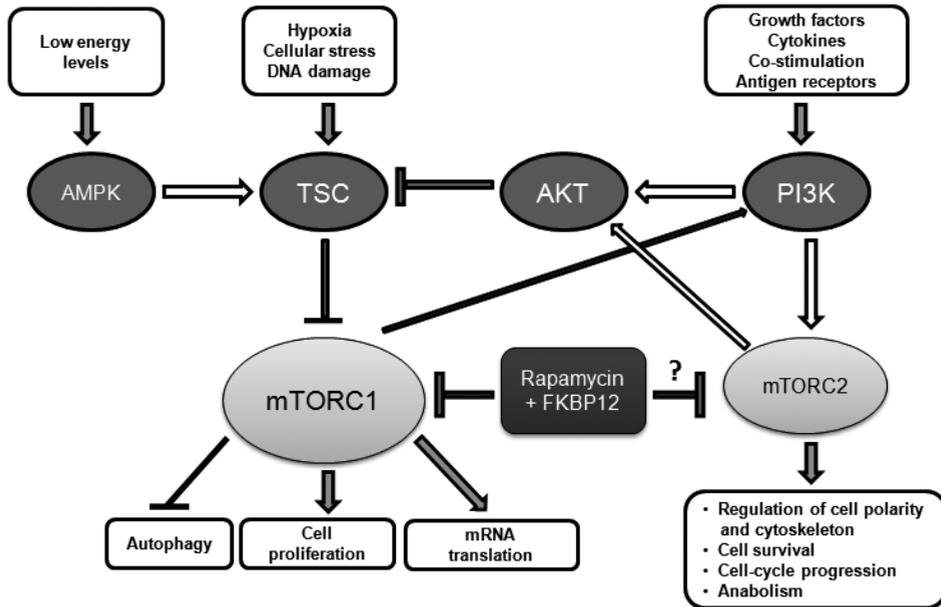


Figure 1. mTOR pathway signaling (simplified). Activation of mTORC starts with activation of the lipid kinase phosphatidylinositol-3-kinase (PI3K) by growth factors, cytokines, co-stimulatory factors or antigen receptors. PI3K, in turn activates Akt. Once activated, Akt phosphorylates and inhibits the tuberous sclerosis complexes (TSC) thereby activating mTOR by releasing it from the inhibitory effects of TSC. TSC can also be directly activated by hypoxia, cellular stress or DNA damage or via AMPK, which in turn is activated by low energy levels, detected as high levels of AMP. Activation of mTORC1 leads to inhibition of autophagy, stimulation of cell proliferation and mRNA translation. mTORC2 is involved in regulation of cell polarity and cytoskeleton and most probably in cell survival, cell cycle progression and anabolism. Rapamycin acts by forming a complex with FK506-binding protein 12 (FKBP-12) which then binds and inhibits mTORC1. Prolonged treatment with rapamycin can inhibit mTORC2 possibly by sequestering the cellular pool of mTOR in a complex with rapamycin-FKBP12 thereby making mTOR unavailable for assembly into mTORC2.

a GFR < 40 ml/min and the presence of proteinuria seem to be contra-indications²⁰.²¹ An early switch to everolimus at 4 months after transplantation improved renal function at one year compared to CNI continuation²². Furthermore, mTOR inhibitors may have beneficial effects on the vessel wall^{23, 24} and have anti-cancer properties. They have been shown to be effective in the prevention of treatment of cancers occurring more often in the immunocompromised renal transplant recipients, like non-melanoma skin cancer²⁵, haematologic malignances²⁶⁻²⁹, Kaposi sarcoma³⁰ and renal carcinoma³¹.

Unfortunately, the use of mTOR inhibitors is associated with a high incidence of adverse effects. Early use of sirolimus or everolimus after transplantation increases the incidence of impaired wound healing³², lymphocele^{33, 34} and the incidence of delayed graft function³⁵. Amongst others, prolonged treatment is

associated with lymphoedema, dyslipidaemia and microcytic anemia^{36,37}, resembling anemia of chronic disease. Another well known side effect is de novo occurrence of - or an increase in proteinuria³⁸. A more severe complication is mTOR associated pneumonitis³⁹, reported as a rare but potential life threatening condition.

Possible explanations for the high incidence of adverse effects accompanying the use of mTOR inhibitors can be postulated. First, since the mTOR pathway is present in almost all cells of the body, it is not surprising that more systems are affected than only the targeted one. Second, although suppressing the adaptive immune response, inhibition of the mTOR pathway enhances innate immunity^{17,40}. This might explain in part the chronic inflammatory state often associated with the use of mTOR inhibitors.

To further clarify the place of mTOR inhibitors in the immunosuppressive drug treatment after renal transplantation, the MECANO trial was conducted, 'Mycophenolate sodium versus Everolimus or Cyclosporine with Allograft Nephropathy as Outcome'. From January 2005 to September 2009, three academic hospitals, the Academic Medical Center, Leiden University Medical Center and the University Medical Center in Groningen, participated in this multi-center randomized controlled trial, studying the effects of early withdrawal of the calcineurin-inhibitor cyclosporine A. Preliminary data were recently published⁴¹. In short, renal allograft recipients, receiving their first or second kidney transplant, were treated with quadruple immune suppression consisting of prednisolone (P), cyclosporine A (CsA), mycophenolate sodium (MPA) and basiliximab. After 6 months, patients were randomized to one of three immunosuppressive drug regimens: P/CsA, P/MPS and P/everolimus (EVL), provided no signs of rejection were detectable in protocol transplant biopsy. Drug exposure of CsA, MPS and EVL was monitored by calculating the Area Under the Curve (AUC) at pre-fixed time-points. The primary outcome was interstitial graft fibrosis and arteriolar hyalinosis. Secondary outcome was, among others, graft rejection. Patients who received a third or fourth transplant were excluded, as were patients with > 50% panel reactive antibodies. The P/MPS arm was prematurely halted because of an increase in severe acute rejection episodes. Substudies of the MECANO trial provided the data for part II of this thesis.

AIM AND OUTLINE OF PART II OF THIS THESIS

Part II will focus on the (side) effects of treatment with everolimus. Pneumonitis, as mentioned above, is one of the potentially most severe complications of mTOR inhibitors. Although several reports have been published on sirolimus-induced pneumonitis, far less is known about everolimus-induced pneumonitis (EIP). **Chapter 6** reports a case-control study in patients of the MECANO cohort treated with everolimus. Incidence, radiologic features and risk factors of EIP are studied in an attempt to elucidate the etiology of this adverse effect.

When mTOR inhibitors were introduced in transplantation, they were used as a calcineurin inhibitor sparing regimen to halt calcineurin associated nephropathy. However an increase in proteinuria was observed after decreasing or withdrawing calcineurin-inhibitors. CNIs have antiproteinuric properties, so it was speculated that the increase of proteinuria resulted from withdrawal of the antiproteinuric properties of CNIs. However, nephrotic range proteinuria was also observed after de novo use of mTOR inhibitors. To clarify this important issue, **chapter 7** compares proteinuria and renal biopsy data, light- and electron microscopy, from patients treated with either everolimus or the calcineurin-inhibitor cyclosporine.

A less well known complication of mTOR inhibitor treatment is discussed in **chapter 8**. It reports a pilot study, initiated after observing an increased incidence of thrombotic events in patients treated with either sirolimus or everolimus, investigating various parameters of hemostasis in patients treated with everolimus or a non-mTOR containing regimen.

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