INTRODUCTION

1.1 Immunosuppressants

Last decade witnessed a global rise in organ transplantations due to advancement in the field of medicine and surgery. Thus, the use of immunosuppressants has increased as they act as chemical agents to aid the acceptance of graft by suppressing the immune system. The apparent rise in the number of transplantations has raised the market demand of these immunosuppressing agents both in value and volume. On the basis of nature and mechanism of action, the immunosuppressant drugs are primarily split into (1) Antibodies (2) Antiproliferative Agents (3) mTOR Inhibitors (4) Glucocorticoids (5) Calcineurin Inhibitors. Fig 1.1 shows the classification of immunosuppressants available in the market.

The history of immunosuppression can be traced back to 1950s when cytostatic drugs were used for neoplastic cell division. Some of such drugs were purine and pyrimidine analogs, azathioprine, cyclophosphamide and folate analogs. They interfered with various processes of immune response including division and differentiation of T cells and B cells. The drawback associated with them was that they also affected the other cells of immune system. Their non-specific action led to the destruction of non-immunocompetant cells which were vital for organism's survival. Though their action as immunosuppressants was satisfactory, they failed to qualify as potent drugs since they lack specificity in action and exhibited large number of side effects.

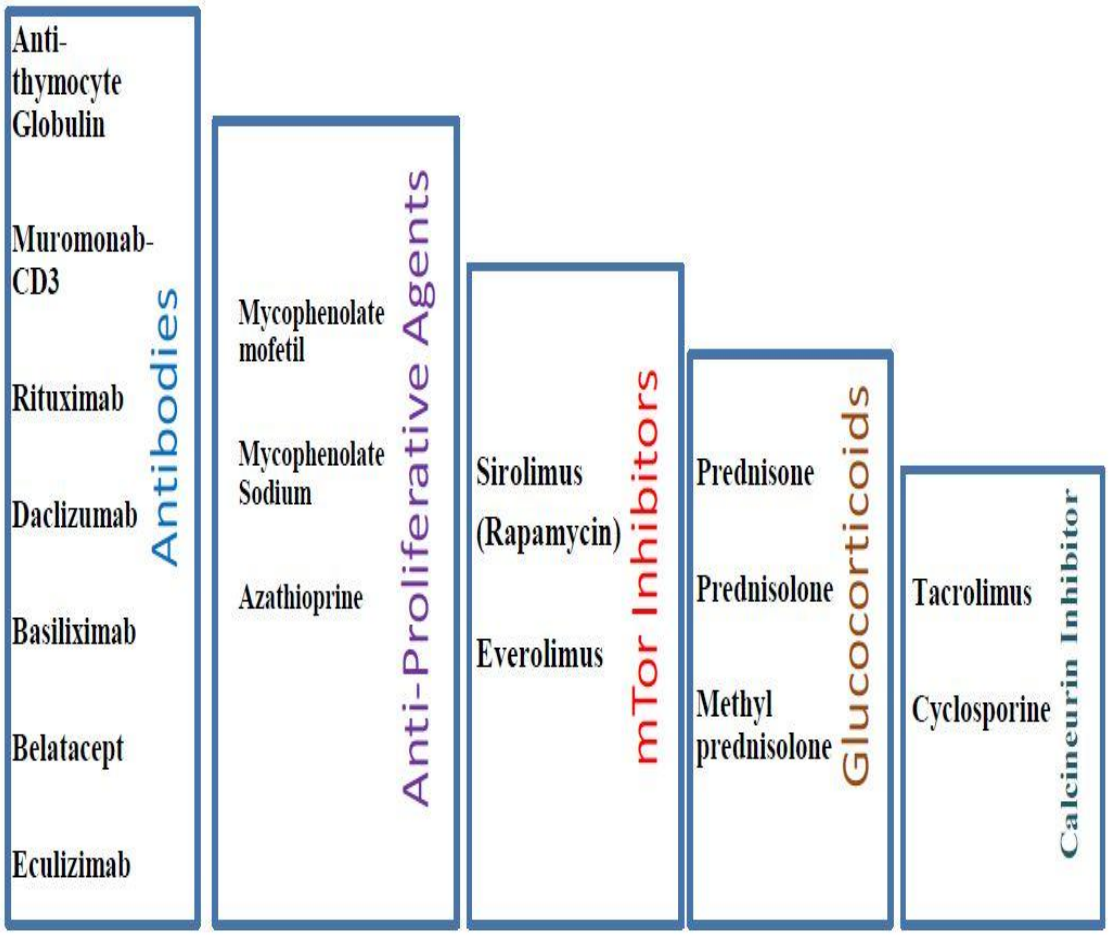

Fig 1.1 Classification of immunosuppressants available in the market

This phase then witnessed the emergence of lymphocytotoxic drugs which were capable of targeting and inhibiting the growth of immune-competent cells such as lymphocytes. Various physical methodologies were adopted for this purpose including removal of spleen, thymus and lymphoid irradiation. Corticosteroids such as cortisols were evaluated as an immunosuppressive agent. They are naturally occurring hormones which cause lysis of lymphocytes, in particular, T-cells. They also interfere with lymphokine production. Preparation of antilymphocyte serum is done by the introduction of human thymocytes in different species such as rabbits. They produced immunoglobulins which were purified and administered intravenously as immunosuppressants. The problem with this process was sensitization against foreign proteins when the immunoglobulins were regularly injected. Thus, it provided a short - term solution for immune rejection.

The second phase of immune suppression was marked by the combinatorial use of different drugs to achieve immunosuppression. This method also emphasized on minimization of the negative effects of these drugs. The most commonly used combination was corticosteroids and azathioprene which increased allograft survival efficiency. Though many side effects also occurred including organ toxicity, diabetes, retarded growth in children, anemia, lethal infections and many others. Autoimmune disorders were treated with steroids while other serious cases used cytostatic drugs.

The third phase of immunosuppressive treatments employed the use of specialized drug which regulates the growth of selective immune-competent cells. This phase evolved by specific targeting of immunologic response and identification of immunogenic stimuli. It focused on the immunocytes cell - signaling system and their interactions. Cyclosporins emerged as first such drug. They acted as calcineurin inhibitors (CNIs) and was validated

by US Food and Drug Administration (US-FDA) in 1983 for heart, liver and kidney transplant [Kolata, 1983]. Another calcineurin inhibitor, tacrolimus was isolated and its immunosuppressive activity was reported which was found to be superior to cyclosporines in its ability to inhibit IL-2 and T-cell activation [Barker *et al.*, 2013, Inamura *et al.*, 1988]. Tacrolimus was found to be effective in heart, liver and kidney transplant and was approved by US FDA in 1994.

In 1975, another team discovered an antifungal compound from the soil sample of Easter Island from an isolated species known as *Streptomyces hygroscopicus*. Based on the place of isolation (Rapa Nui island), the compound was named as rapamycin. Later, it was found to be a very potent immunosuppressive agent. As immunosuppressant rapamycin was found to act as an inhibitor of a kinase protein known as mammalian Target of Rapamycin (mTOR).

1.2 Approval of rapamycin as immunosuppressant

Rapamycin was evaluated as immunosuppressive drug for transplantations in different animal models [Calne *et al.*, 1989, Morris *et al.*, 1989].

Phase I clinical trial was initiated in 1993, when rapamycin was administered to transplant patients. The effect of increasing dose concentration on the patients was analyzed with respect to safety. Pharmacokinetic data were collected for the liquid formulation which was administered orally and was found to show few side effects [Murgia *et al.*, 1996]. In phase II studies rapamycin-cyclosporinA combination was evaluated which established the drug as safe and efficient with fewer events of acute rejections [Kahan *et al.*, 1998]. Finally,

phase III trials were conducted on a large, random and blind set of populations. The results were satisfactory. Therefore, in September 1999, sirolimus (commercial name of rapamycin) was validated by US-FDA to be used in combination with cyclosporins and steroids for renal transplant. Then in 2000, the approval was given by European agency for use of sirolimus as a substitute of calneurin inhibitors as maintenance treatment. Later in year 2003, FDA permitted the reduction in dose of cyclosporine by substituting it with an increased dose of sirolimus. Use of sirolimus in coated stents for angioplasty was also approved.

1.3 Rapamycin as compared with other immunosuppressants

Rapamycin differs with other calcineurin inhibitors like tacrolimus and cyclosporins in the the mechanism of action such that acts more selectively on immune-competent cells. CNIs exhibit their immunosuppressive action by inhibition of IL-2 production and other interleukins, whereas rapamycin action initiates during downstream signaling of IL-2 production [Andersson *et al.*, 1992, Kay *et al.*, 1991]

It was found to be better than other CNIs as it reduced the risks associated with organ transplant such as hypertension, nephrotoxicity and neurotoxicity [Paghdal *et al.*, 2007, Ziolkowski *et al.*, 2003].

Another report suggests that many transplant recipients suffer from hemolytic uremia syndrome (HUS). Such patients are at risk of systemic disease which may be lethal [Ducloux *et al.*, 1998]. Such risk was found to enhance if CNIs are used. A study found that replacement of CNIs with rapamycin can reduce the problem of HUS without affecting the graft tolerance [Edwards *et al.*, 2002].

Rapamycin was also suggested to act as an alternative of CNIs in cases of delayed graft function (DGF) of renal allografts. CNIs may increase the problem due to the associated nephrotoxicity and risk of vasoconstriction [Gonzalez *et al.*, 1990].

CNIs are also found to be associated with the occurrence of gout in transplant patients due to an increase in uric acid level. Rapamycin was suggested to lower the uric acid level and thus reduce the chance of gout [Dupont *et al.*, 2003].

A study also suggests that other immunosuppressant mycophenolate mofetil is associated with haematological and gastrointestinal problems which lead to discontinuation of drug in many cases. Rapamycin is proposed to act as an alternative in such cases [Sollinger, 1995].

1.4 Other roles of Rapamycin

Rapamycin has several functions as a therapeutic agent. Some of the roles of rapamycin have been compiled in Fig 1.2.

Studies have also been conducted for use of sirolimus in conditions like Kaposi's sarcoma, psoriasis and tuberous sclerosis. The drug was also found to be potent in the treatment of other disorders like dermatitis [Trautmann *et al.*, 2001], wound healing [Ong *et al.*, 2007] and Muir-Torre syndrome [Paghdal *et al.*, 2007, Schwartz *et al.*, 1995].

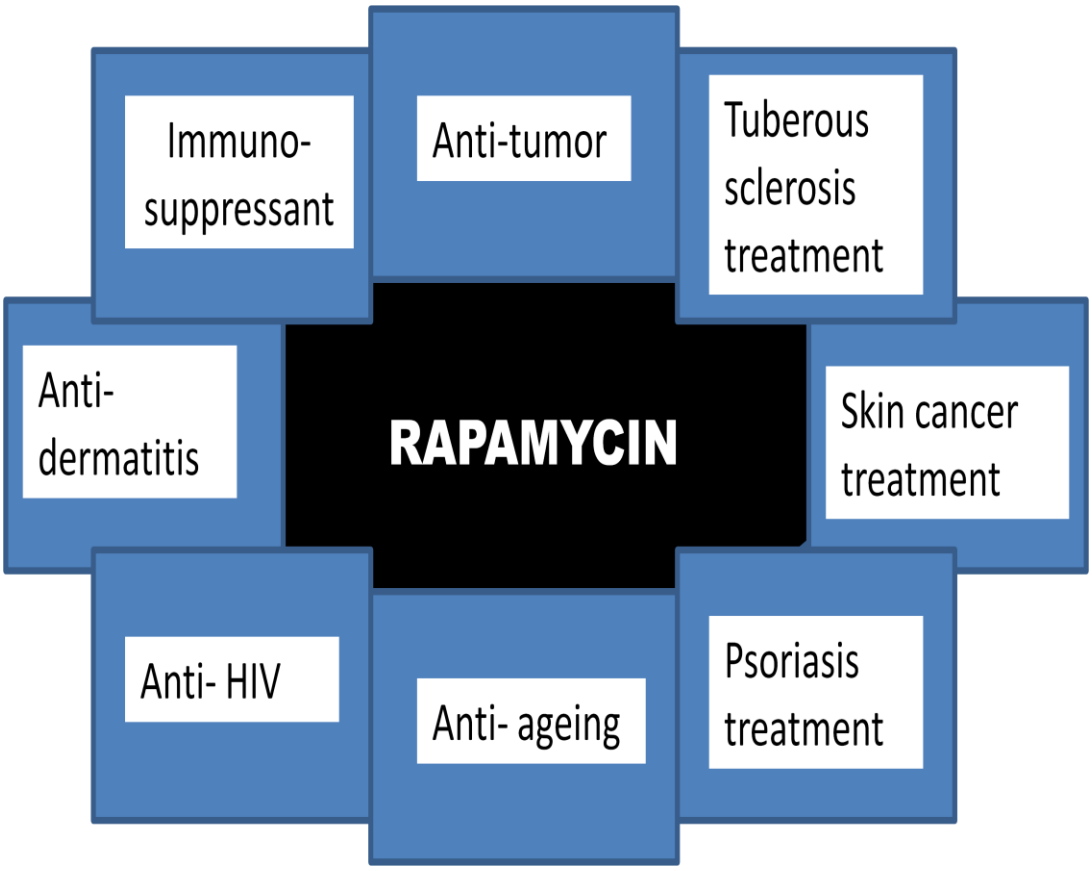

Fig 1.2 Therapeutic functions of rapamycin

1.4.1 Treatment of tuberous sclerosis

As an mTOR inhibitor rapamycin promotes the production of unphosphorylated S6K which leads to cell cycle arrest. Also, rapamycin reduces vascular endothelial growth factor (VGEF) and checks the growth of vascular tumors which are found to be associated with tuberous sclerosis [Brugarolas *et al.*, 2003].

1.4.2 Reduction in occurrence of skin cancer

A report states that increased incidences of melanoma and non-melanoma skin cancer have been witnessed in patients after the kidney transplant. When sirolimus monotherapy was compared with combination treatment, it was found that with monotherapy, the frequency of occurrence of skin cancer was significantly lower. Also, no compromise in graft acceptance was observed [Campistol *et al.*, 2006].

1.4.3 Treatment of Psoriasis

Psoriasis which is characterized by proliferation of keratinocytes and infiltration of neutrophils was found to be treated by the use of rapamycin. Thus, patients a receiving combination of sirolimus-cyclosporins were found to be treated for psoriasis as compared to monotherapy of cyclosporine [Reitamo *et al.*, 2001].

1.4.4 Anti-tumor effect

Rapamycin has gained importance as an anti-tumor agent also. It was proposed that rapamycin induces apoptosis in malignant cells selectively. This result was obtained as rapamycin acts as a Target of rapamycin (TOR) inhibitor. TOR has a crucial role in the

maintenance of transformed cells as the upstream and downstream pathways deregulate during cancers [Bjornsti *et al.*, 2004]. Rapamycin was found to be effective against cell lines of the central nervous system, melanocytes, liver, lymphoid, connective tissue, kidney and osteoblastic cells. The action was found to be effective against transformed cell lines [Sehgal, 2003]. Rapamycin was reported to act as a potent anti-cancer agent in case of ependymoblastoma, melanocarcinoma, colon and mammary tumors [Douros *et al.*, 1981]. Rapamycin was also found to exhibit anti-tumor function in head and neck squamous cell carcinoma (HNSCC) in animals [Hu *et al.*, 2011].

1.4.5 Anti-ageing property

Rapamycin was suggested to extend lifespan in mammals. Rapamycin was first established as an anti-ageing drug in a study conducted on mice [Harrison *et al.*, 2009, Neff *et al.*, 2013]. It was found that administration of rapamycin for three months enhanced the life-expectancy of the subjects which were middle - aged mice [Bitto *et al.*, 2016]. Further study revealed that anti-ageing effect of rapamycin was due to its property of suppressing cancers in mammals. Moreover, it was also found to affect the cognitive impairments which were age- related [Anisimov *et al.*, 2011].

1.4.6 Potent role against Human Immuno-deficiency Virus (HIV)

Rapamycin was found to inhibit the gene expression of CCR5 chemokine receptor on CD^{4+} T cells. The density of these CCR5 receptors is an important parameter for HIV type 1 infection. Rapamycin reduces the density of these receptors and thus shows antiviral activity against HIV virus [Heredia *et al.*, 2007].

1.5 Use of rapamycin in coronary stents

Rapamycin is used for coating the coronary stents as it exhibits inhibition of vascular smooth muscle [Gallo, 1999]. This property of rapamycin was employed to cure coronary artery disease. Studies showed that the use of rapamycin or sirolimus in coating coronary stents tend to reduce restenosis rate by preventing neo-intimal proliferation [Sousa *et al.*, 2001]. A study done on sirolimus coated coronary stent receiving randomized population showed that there was a significant reduction in restenosis rate when compared with those receiving normal stent [McAlister *et al.*, 2002].

1.6 Nano-rapamycin

Rapamycin has become a promising drug for treatment of many disorders including graft rejection, tumors, restenosis, inflammation and coronary diseases. Major roadblocks associated with rapamycin as a therapeutic agent is its poor water solubility, limited oral bioavailability and non-specific distribution. Recent advances in the field of nanotechnology have provided a solution for sirolimus delivery. To facilitate this technique, different nano-carriers have been designed such as micelles, liposomes, polymeric, albumin and magnetic nanoparticles [Haeri *et al.*, 2017]. Fu *et al.* prepared a nanosized microcapsule containing chitosan, poly lactic acid and tripolyphotspate for entrapment of rapamycin which enhanced with increasing the concentration of rapamycin [Fu *et al.*, 2010]. In another study, the osteointegration of titanium - based implants was improved by loading rapamycin onto nano-hydroxyapatite layer coated titanium implants. The results showed improved osteogenesis as well as phosphorylation of mammalian target of rapamycin (mTOR) [Liu *et al.*, 2017].

1.7 Side effects associated with rapamycin

Though rapamycin was found to be associated with significant number of therapeutic uses still some adverse effects were also observed in some cases. Regular administration of rapamycin causes side effects such as diarrhea, somatitis, headaches, poly-arthralgia and epistaxis. Rapamycin was found to be associated with dose dependent thrombocytopenia which was recovered as treatment proceeded [Murgia *et al.*, 1996]. Rapamycin was also reported to cause hyperlipidemia in renal transplant patients after two months of administration [Almond *et al.*, 1993]. Another problem associated with rapamycin dosing to renal implant patients is over immunosuppression. It was noted that post- transplant the occurrence of infections such as herpes simplex infections and pneumonia increased. Also, the chance of neoplasia and lympho-proliferative disorder increased [Groth *et al.*, 1999].

1.8 Manufacturers of rapamycin

Rapamycin is marketed under various trade names by different manufacturers. Table 1.1 provides the list of different manufacturers working as key players in commercialization of rapamycin.

Table 1.1 Manufacturers of Rapamycin

Year of Discovery	Year of approval	Trade Name	Manufacturers
1975	1999	Sirolimus®	Pf Prism Cv
		Temsirolimus ®	Dr Reddys Labs Ltd
		Rapamune®	Wyeth Ltd.
		Siromus®	Zydus Pharms USA Inc
		Rapacan®	Biocon

After receiving approval by the US-FDA in1999, rapamycin was first commercialized by Wyeth (now purchased by Pfizer). Rapamycin (generic name: sirolimus) was approved by the US- FDA for use in the renal transplant in 1999 and for treatment of Lymphangioleiomyomatosis (LAM) in 2015 [Gupta *et al.*, 2015]. Currently, rapamycin formulated in the form of an oral solution and as a tablet (each containing 1 or 2 mg rapamycin) [Madke, 2013]. With a large number of therapeutic applications associated with rapamycin, the global market tends to witness considerable growth.